

# What kind of drugs should we develop

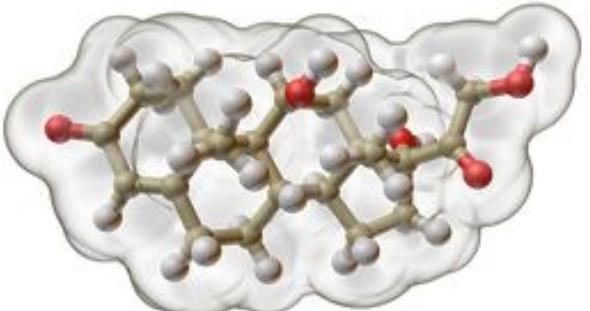
*Mathematical and Computational Biology in Drug Discovery  
(MCBDD) Module III*

*Dr. Jitao David Zhang  
April 2024*

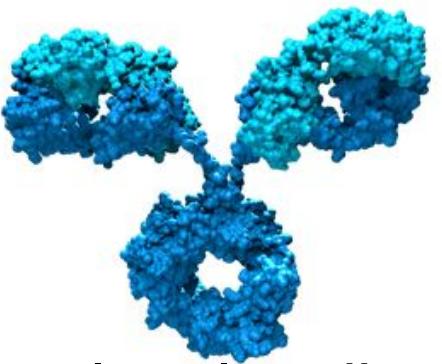
# Overview

- Essentials of modalities
  - Small molecules: classical, protein degrader, RNA modulator
  - Large molecules: classical, DUTA-Fabs, protein design
  - Antisense oligonucleotides: siRNA, shRNA, ASO
  - Gene and cell therapy
- Three case studies:
  - Success stories:
    - [Small molecules] SMA (Evrysdi/Risdiplam and Nusinersen)
    - [Antisense] patisiran ([KEGG DRUG](#)) and givosiran ([DrugBank](#), [structure available at EMA](#))
    - [Offline read] mRNA vaccine (MIT Technology Review)
    - Turning failure into successes: [Multispecific drugs] Thalidomide, PROTAC, degraders
    - [Antibody] Cancer immunotherapy (CTLA4, PD1)
    - [Gene and Cell therapy] CAR-T
  - Challenges
    - [Antisense] HTT (Tominersen)
    - Difference between genetic and enzymatic inhibition

# A zoo of modalities



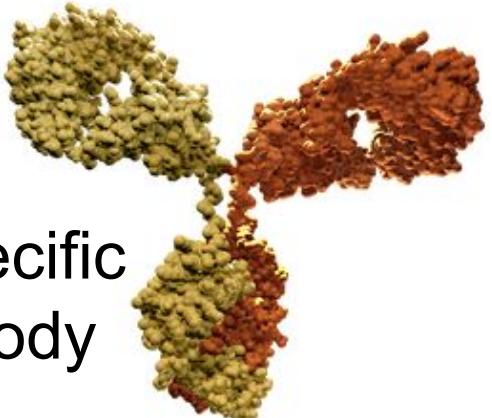
Small molecule



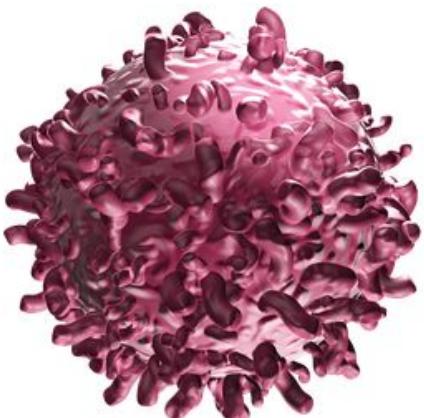
Monoclonal antibody



Oligonucleotides



Bispecific  
antibody



Chimeric  
Antigen  
Receptor  
(CAR)  
T-cells



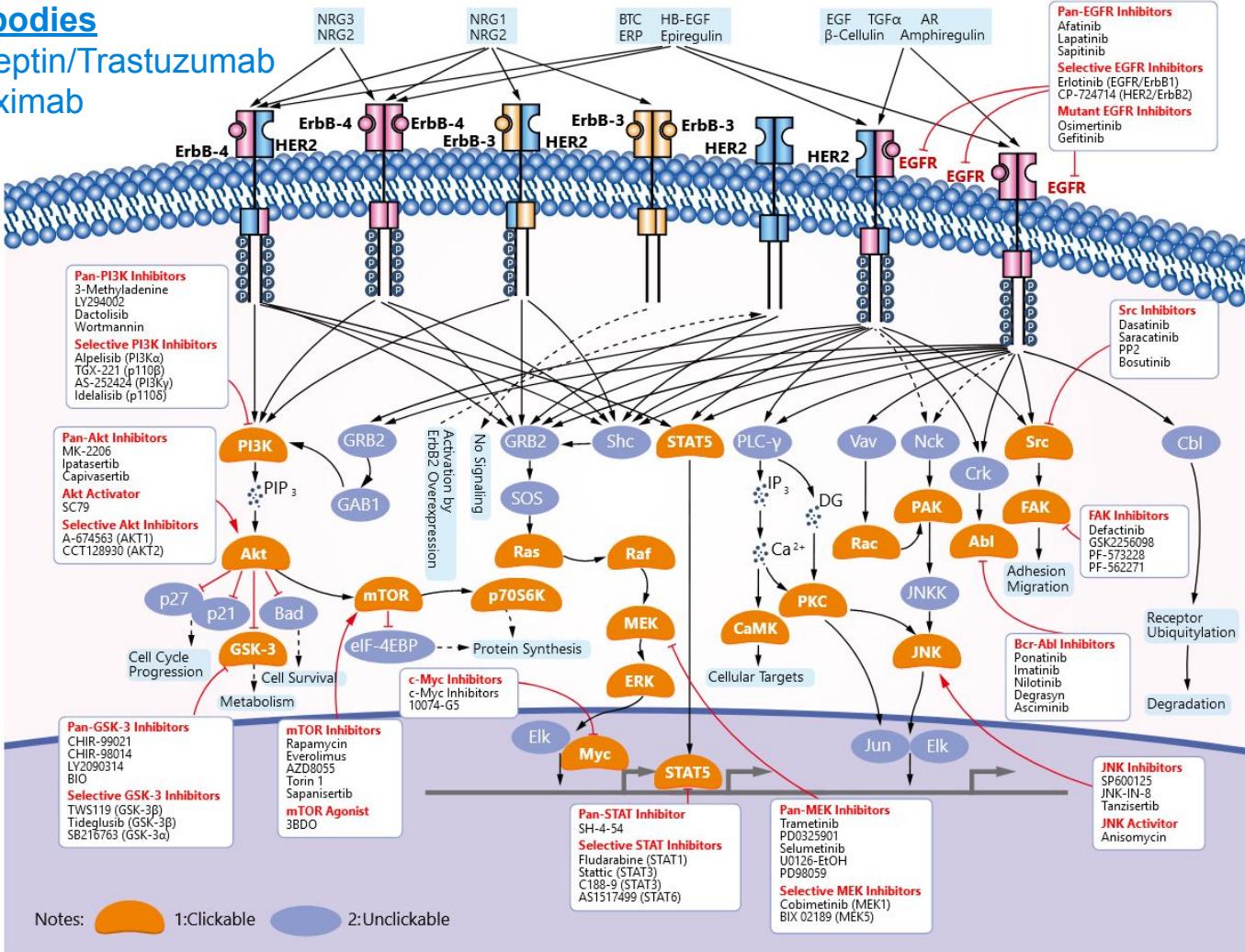
mRNA vaccines

# Multiple modalities can target the same biological process

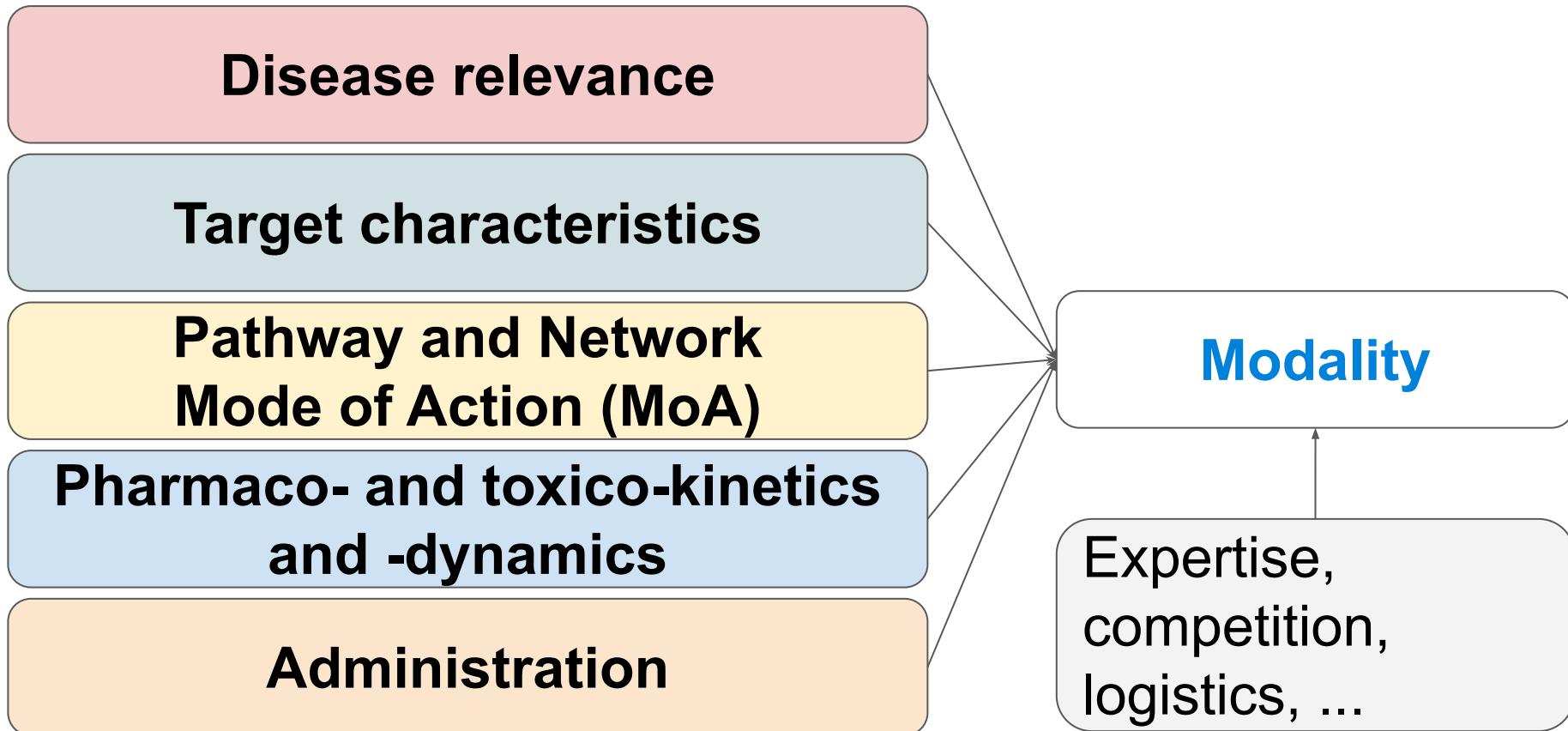
An example: the epidermal growth factor receptor (EGFR) pathway

## Antibodies

Herceptin/Trastuzumab  
Cetuximab



# Criteria to choose a modality

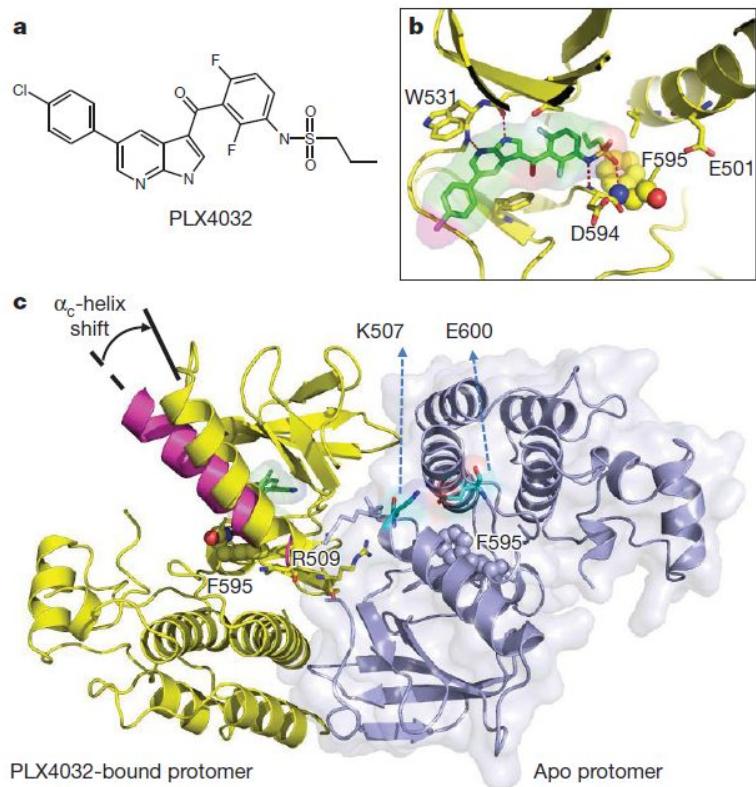


# Characteristics of therapeutic modalities

Modality	Cause of disease at the protein level		Molecular target	Protein target localization			Delivery
	Reduction or loss of function	Excessive or detrimental function		Extracellular	Plasma membrane	Intracellular	
Small molecule	●	●	DNA → RNA → Protein	●	●	●	Oral Injection Inhaled
Protein replacement	○			○	○	○	○
Antibody		●		●	●		●
Oligonucleotide therapy	○	○	○	○	○	○	○
Cell and gene therapy*	●		●	●	●		●

# Classical small molecules: an example from AMIDD

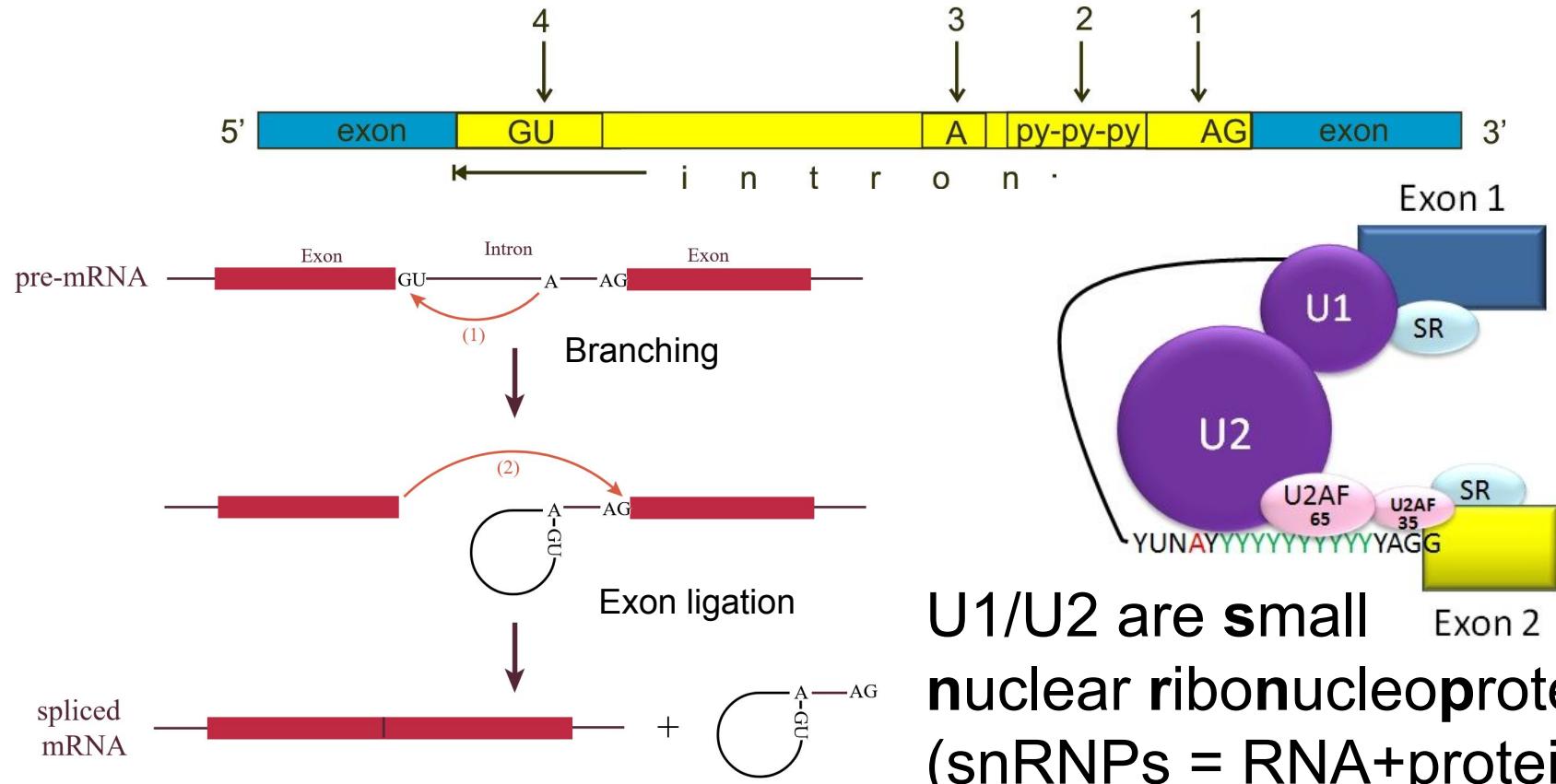
- Vemurafenib (Zelboraf, PLX4032)  
**V600E mutated BRAF inhibition**
- Lock and key: an oversimplified yet powerful metaphor, first proposed by Emil Fischer



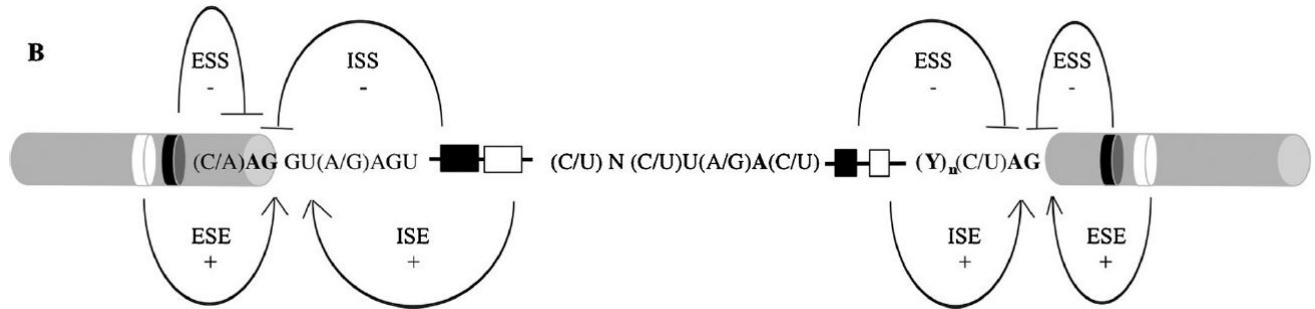
# Facts about Spinal Muscular Atrophy (SMA)

- SMA is caused by a defect in a gene called *SMN1*. People with SMA have reduced levels of the SMN protein.
- When SMN protein levels are reduced, motor neurons are unable to send signals to the muscles, causing them to become smaller and weaker over time.
- Depending on the severity, or type of SMA, people with the disease will have difficulties moving, eating, and in some cases breathing, making them increasingly dependent on parents and caregivers.
- A short movie: <https://www.nejm.org/doi/full/10.1056/NEJMoa2009965>

# Spliceosome: the splicing machinery



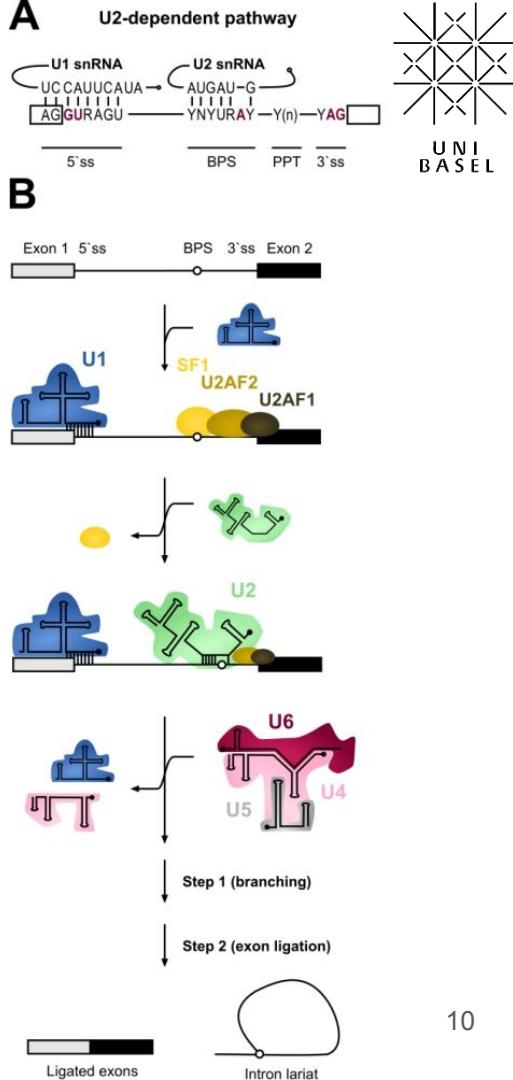
# Splicing in action and under regulation



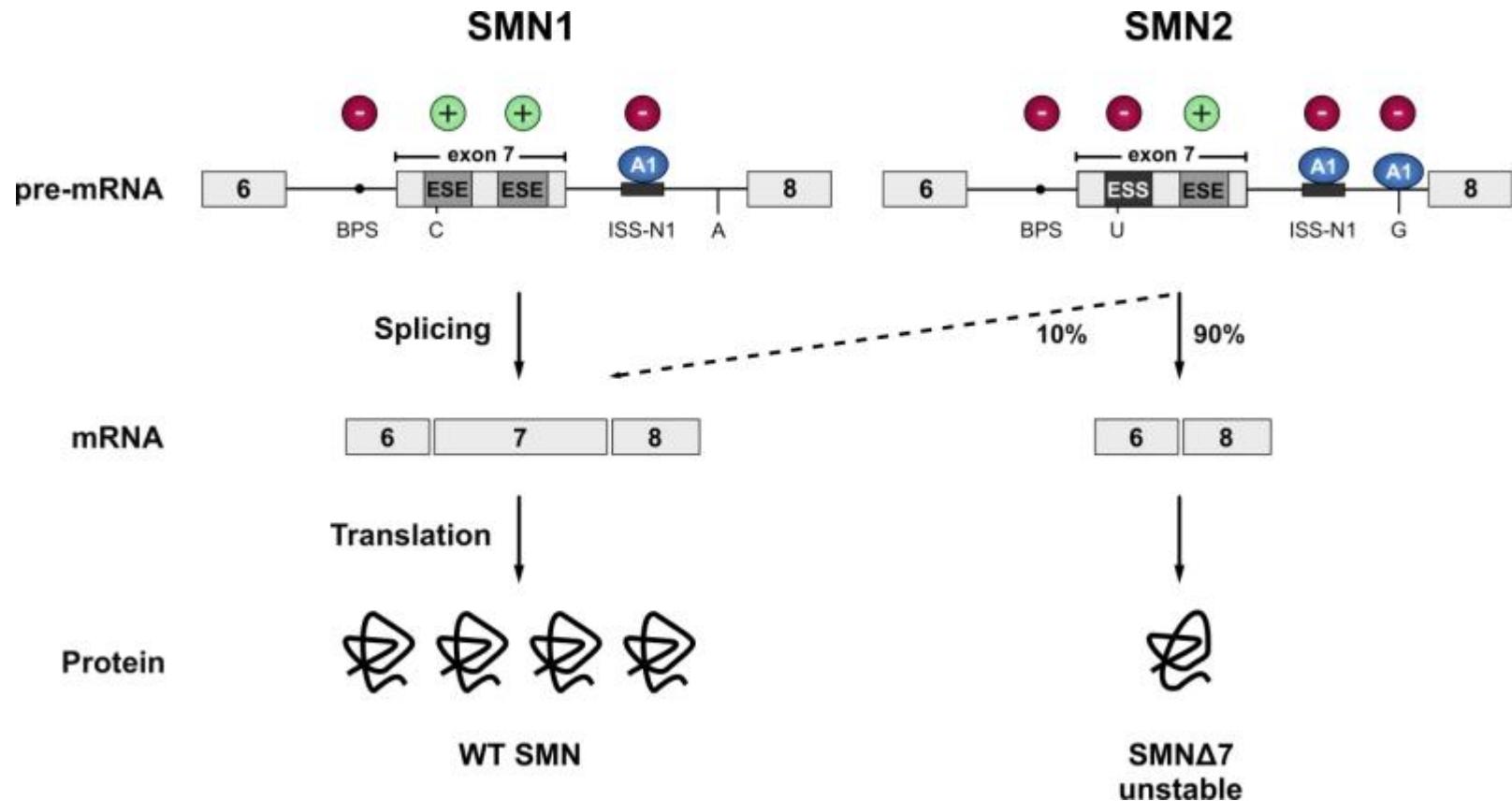
ESS=exon splicing silencer; ESE=exon splicing enhancer;

ISS=intron splicing silencer; ISE=intron splicing enhancer.

BPS=branch point sequence; PPT=polypyrimidine tract (C/U);

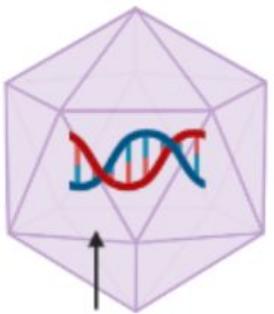


# Different splicing of SMN1 and SMN2

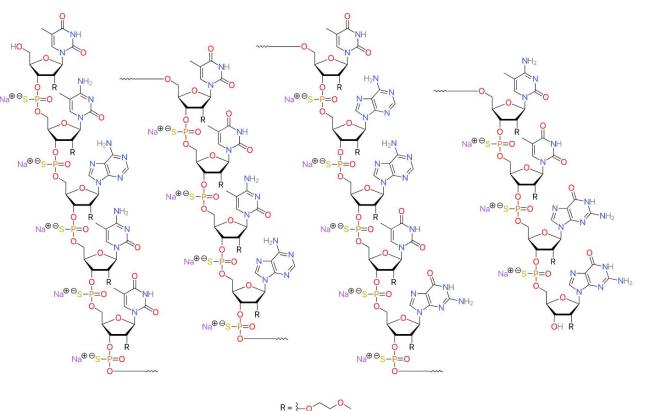


# One Disease, Three Drugs

AAV9 capsid

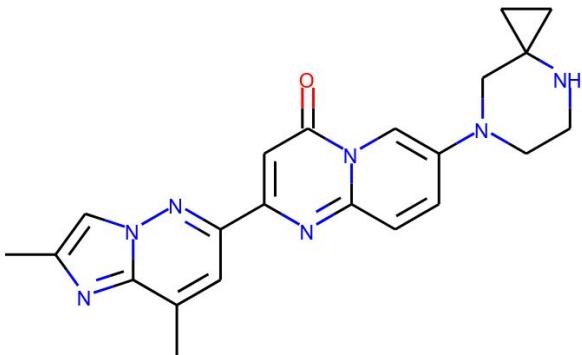


SMN1 gene



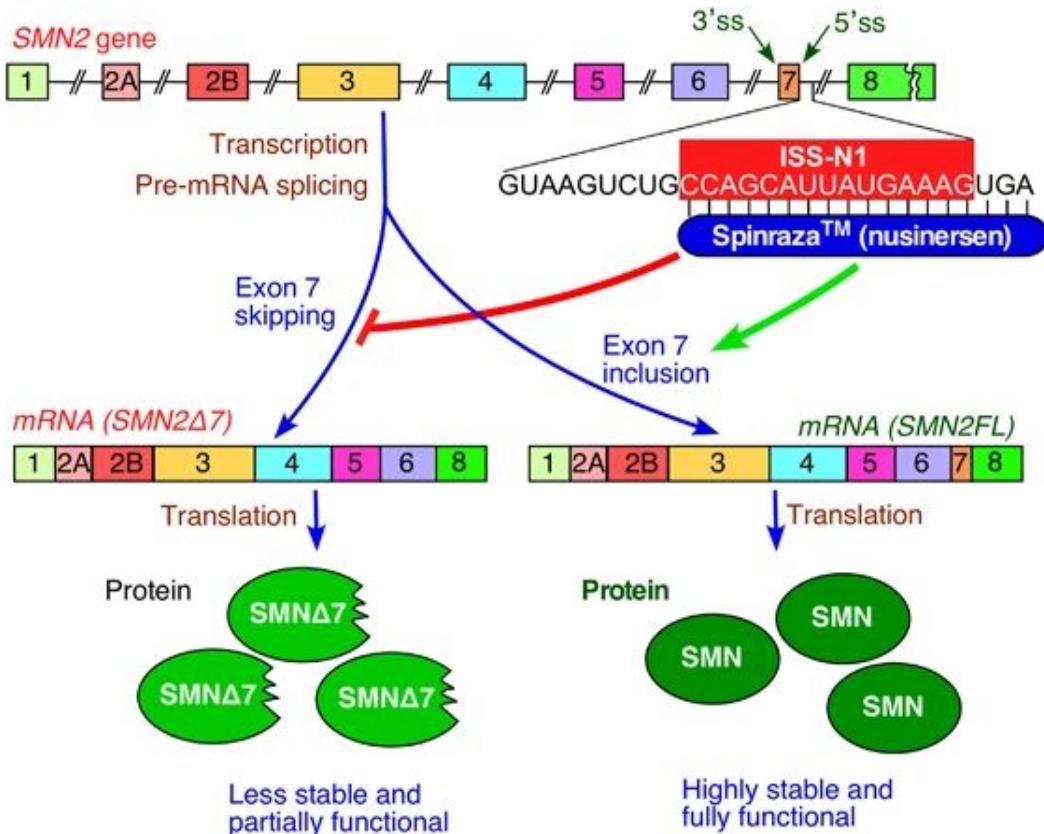
Onasemnogene  
Abeparvovec/  
Zolgensma

Nusinersen sodium/ Spinraza  
([CHEMBL3833342](#))

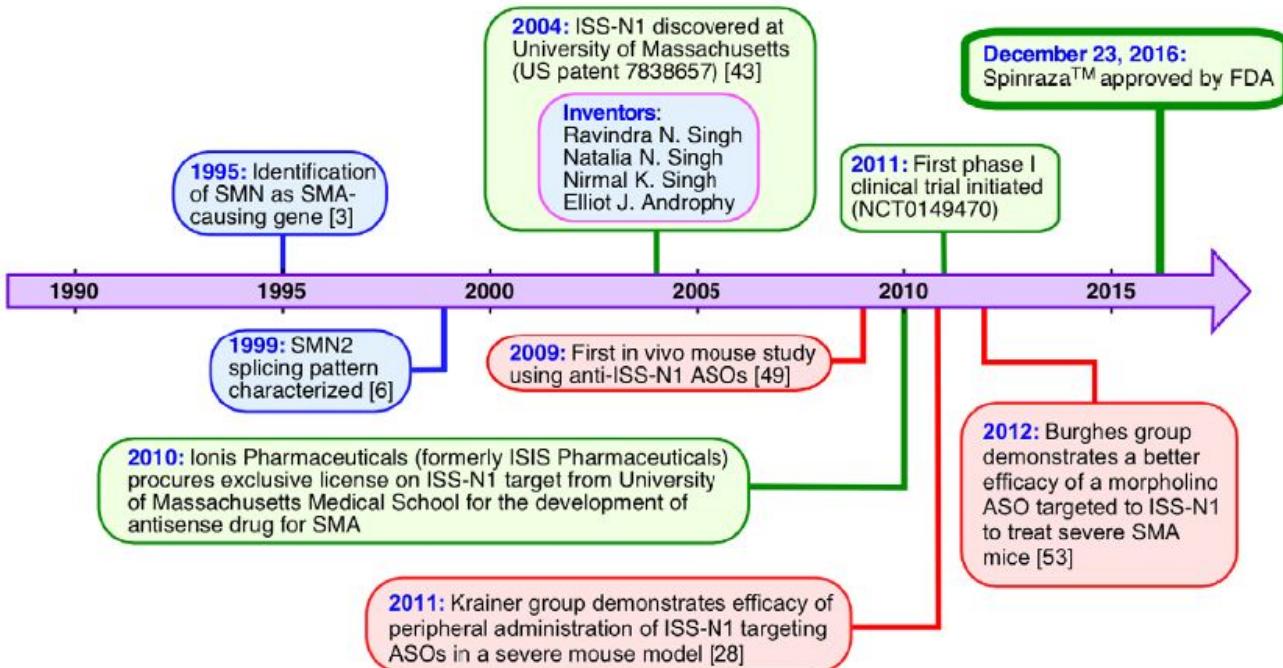


Risdiplam/ Evrysdi  
([CHEMBL4297528](#))

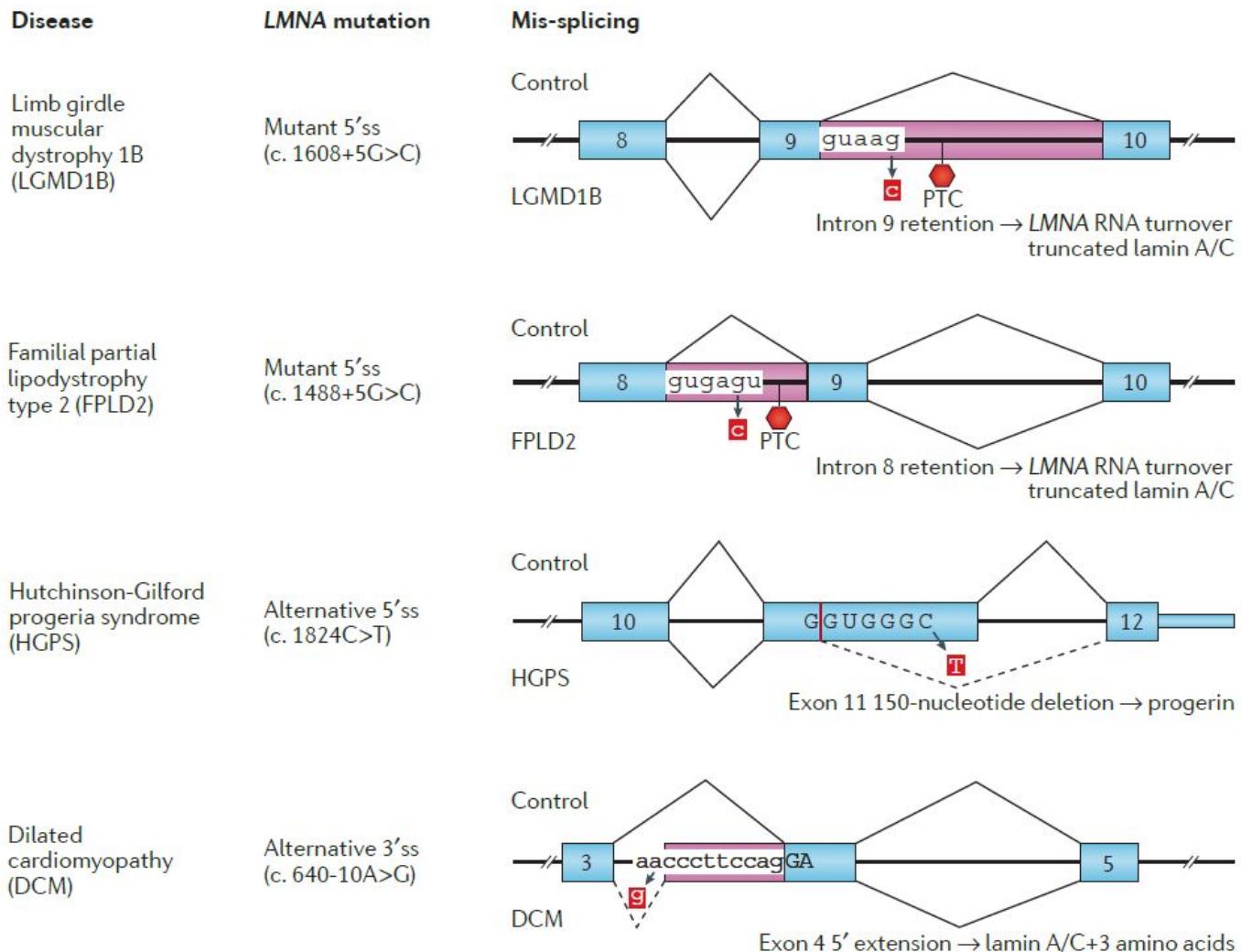
# How Spinraza (nusinersen) works



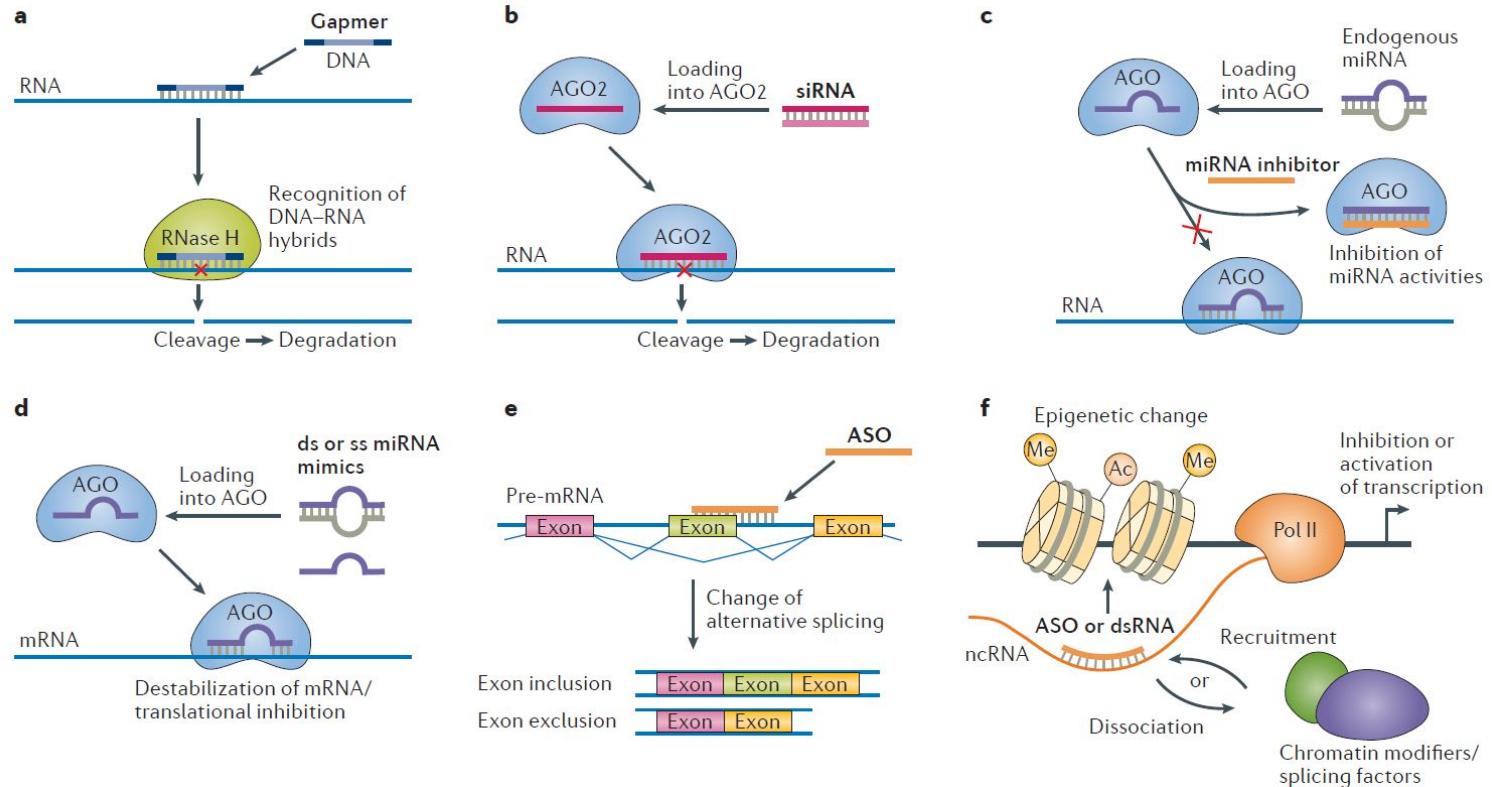
# It takes 21 years to go from a molecular model to a population model



# Splicing modifying oligo-nucleotide and other RNA therapeutics have strong disease relevances

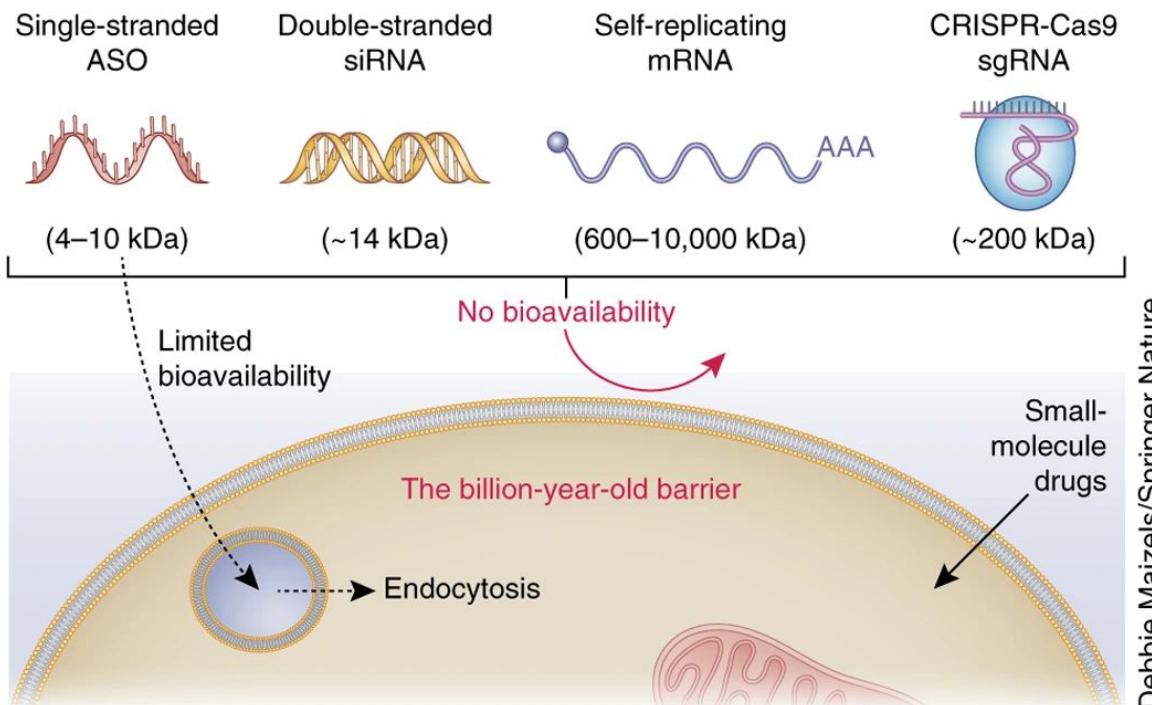


# Regulating RNA levels or splicing with ASOs and duplex RNAs



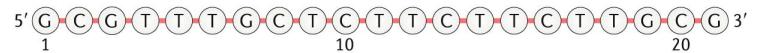
# The four-billion-year-old barrier to RNA therapeutic

- Too large and charged to pass lipid bilayers
- Degradable by RNases
- Rapid clearance from liver and kidney
- Immunogenicity
- Endocytosis
- Delivery into organs other than liver and eye

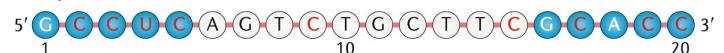


# Chemistry of oligonucleotides evolves with time

a Fomivirsen



b Mipomersen



c Inotersen



d Eteplirsen



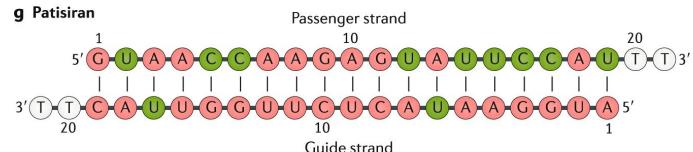
e Golodirsen



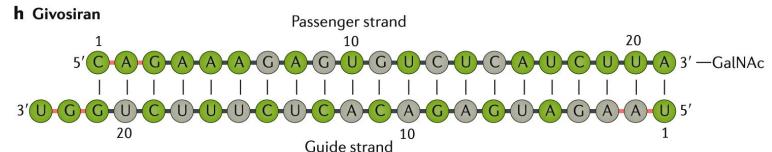
f Nusinersen



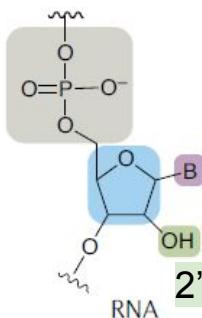
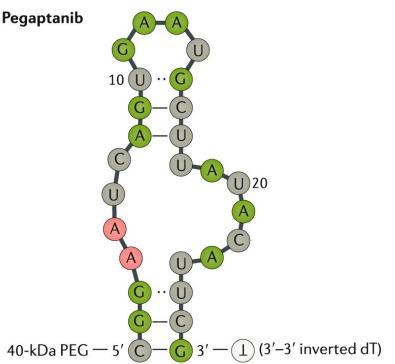
g Patisiran



h Givosiran



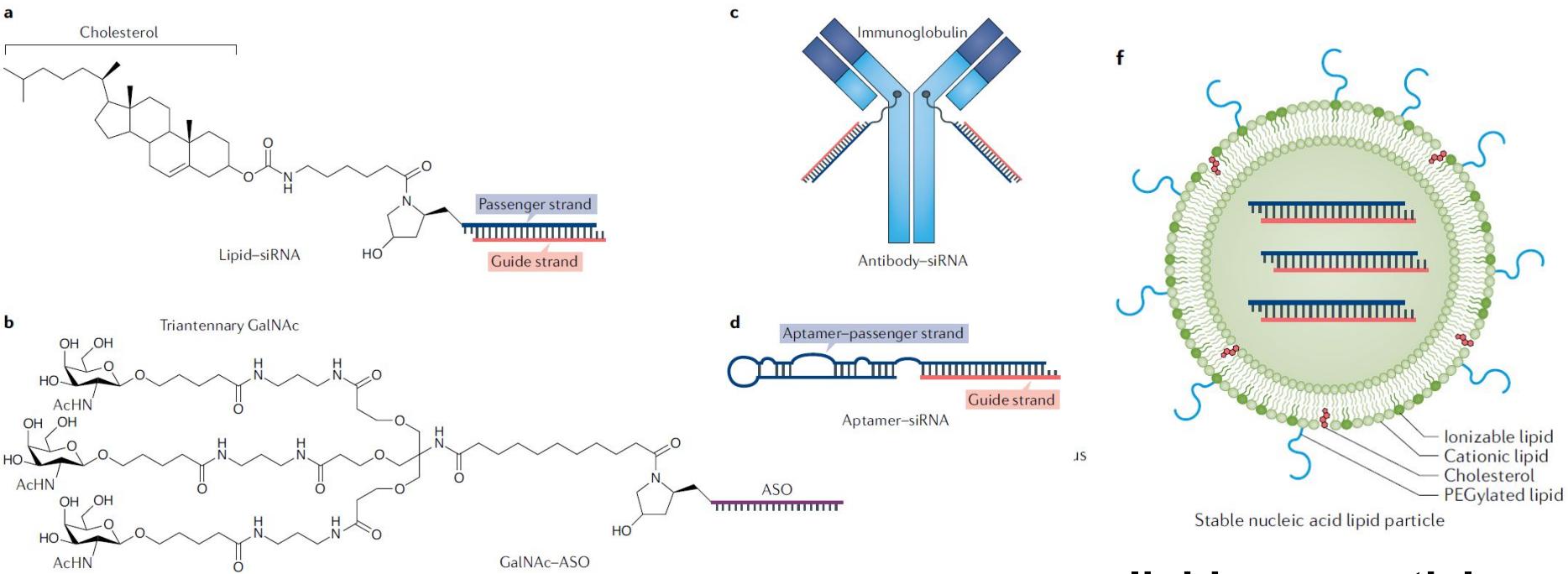
i Pegaptanib



- (N) DNA
- (N) RNA
- (N) PMO
- N 2'-O-methoxyethyl
- N 2'-O-methyl
- N 2'-Fluoro
- Y 5-Methyl pyrimidine
- Phosphorothioate
- Phosphodiester

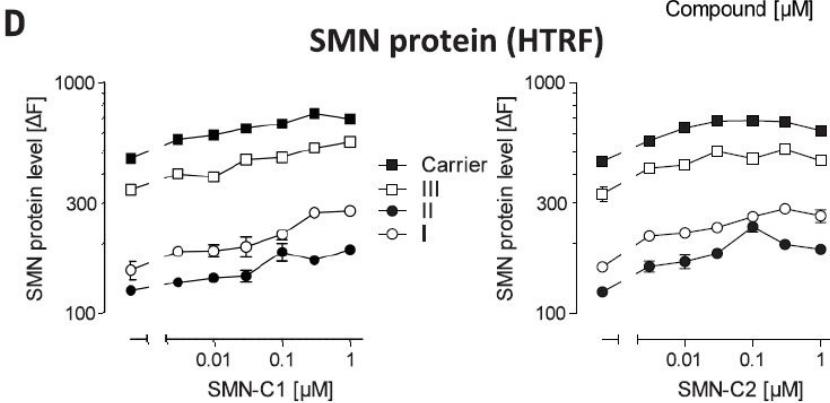
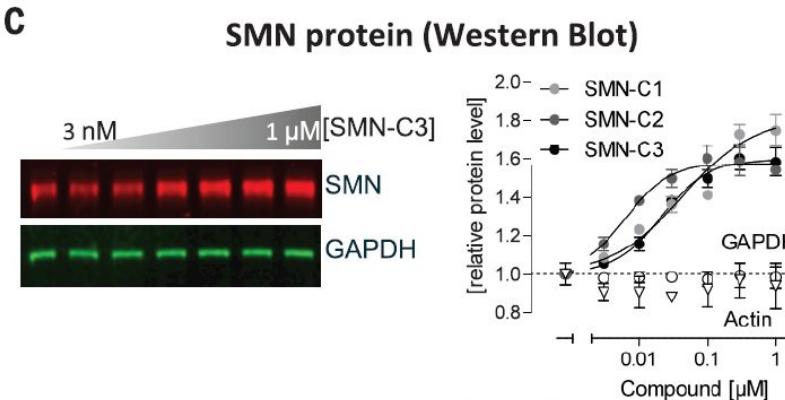
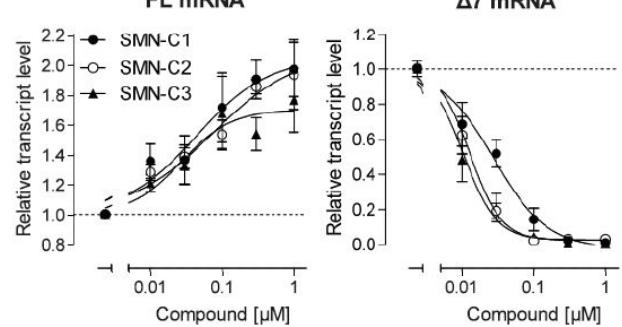
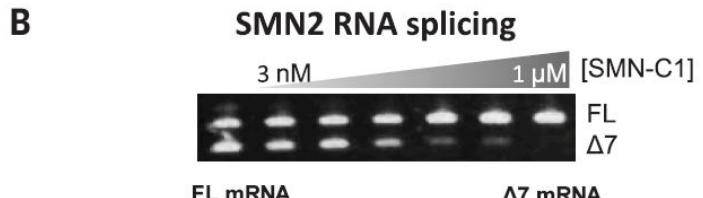
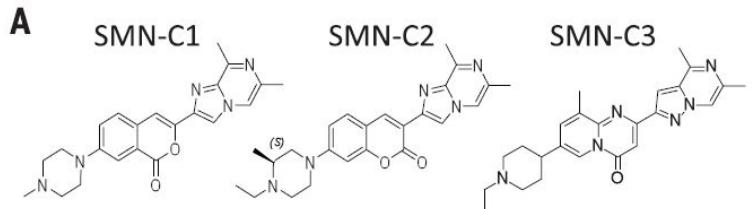
PMO=phosphorodiamidate morpholino oligomer

# Delivery systems of antisense oligonucleotides



## lipid nanoparticles

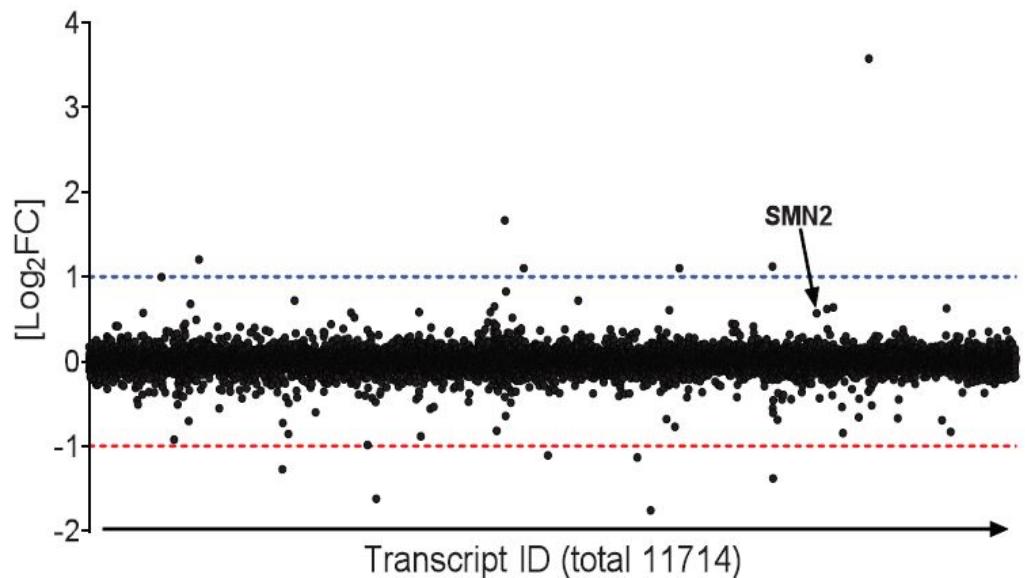
# Small molecules as RNA splicing modifiers



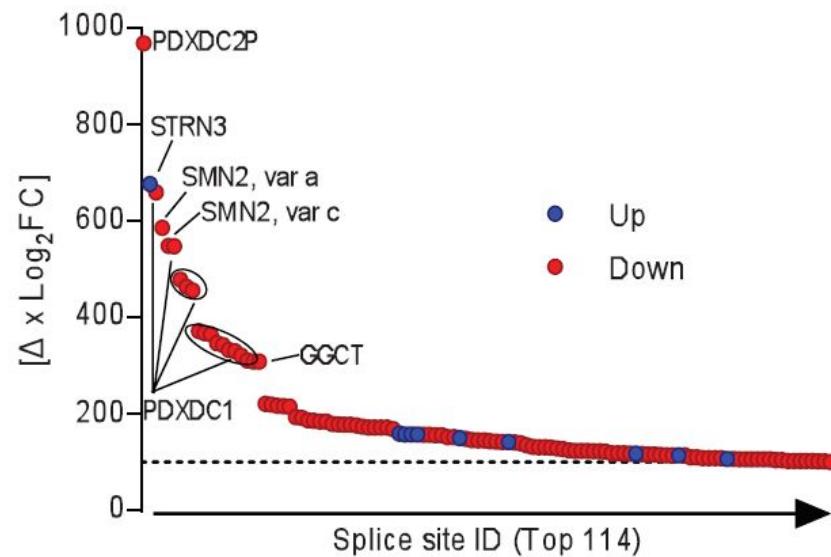
# RNA sequencing confirms the specificity of SMN-C3

**A**

Transcriptional changes by SMN-C3

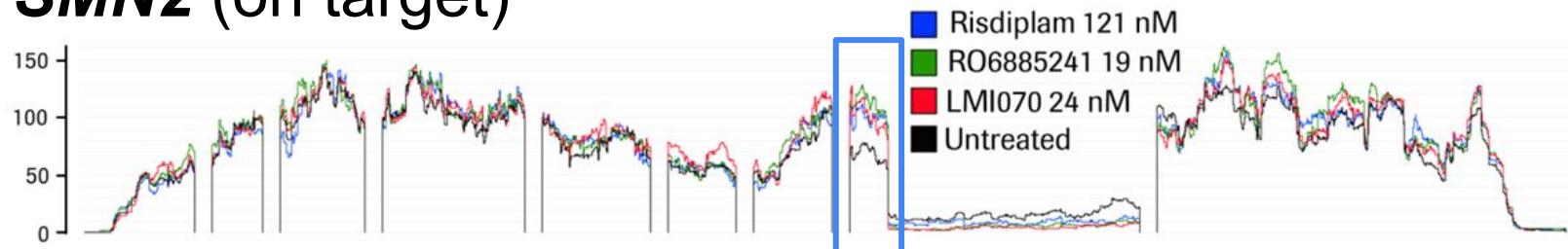

**B**

Splicing regulation by SMN-C3

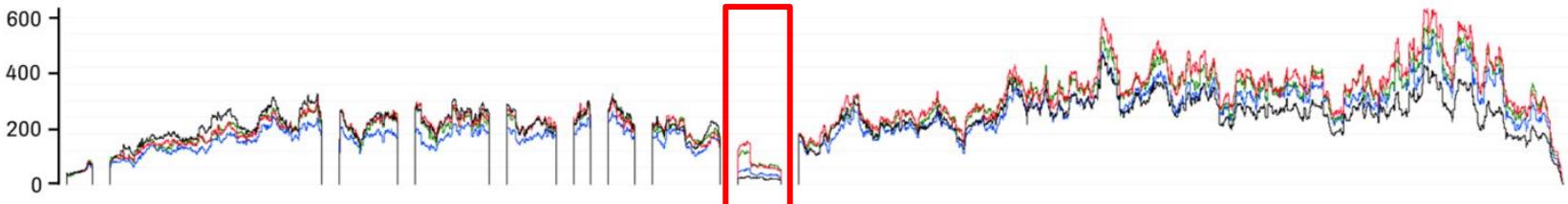


# RNA sequencing confirms the specificity of SMN-C3

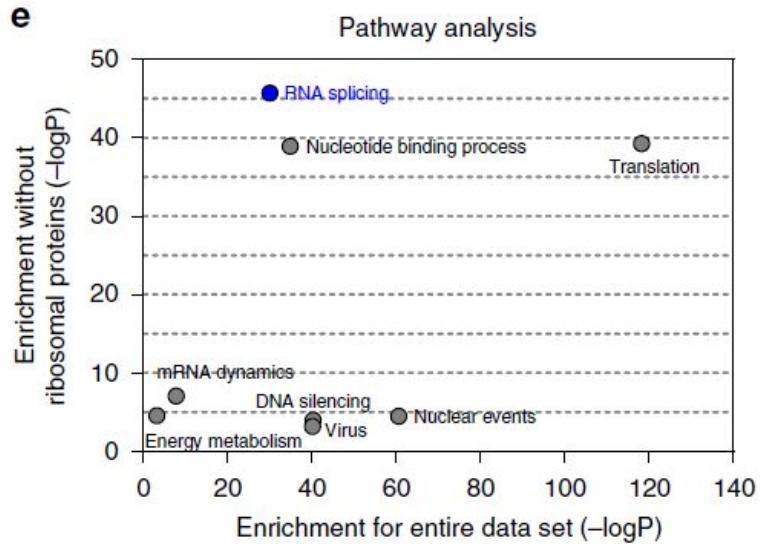
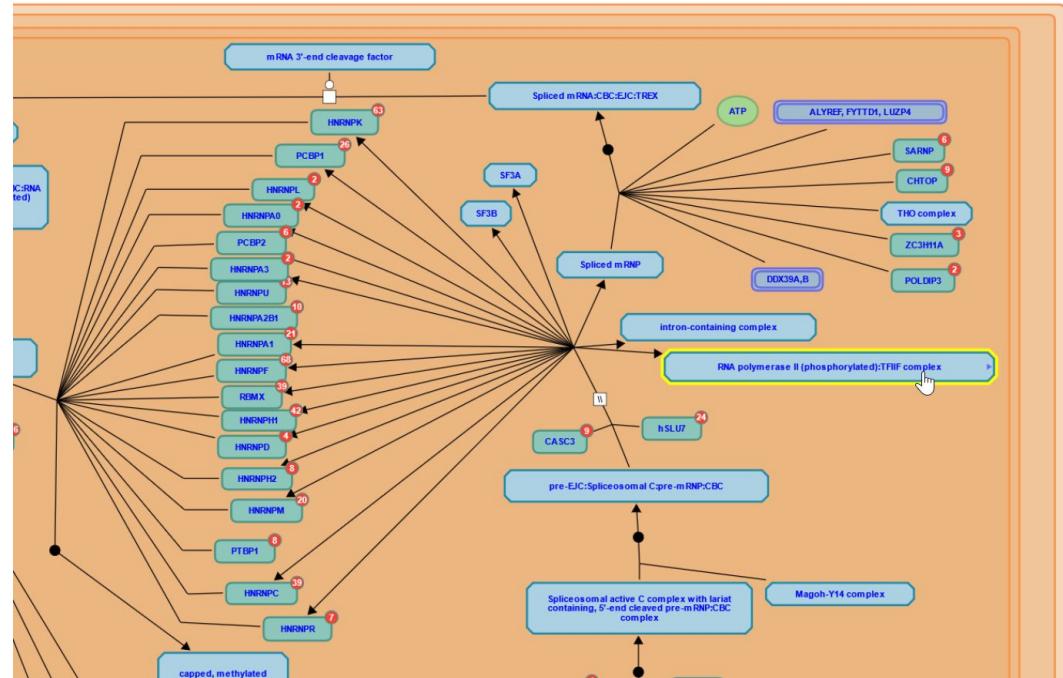
***SMN2*** (on target)



***FOXM2*** (off target)

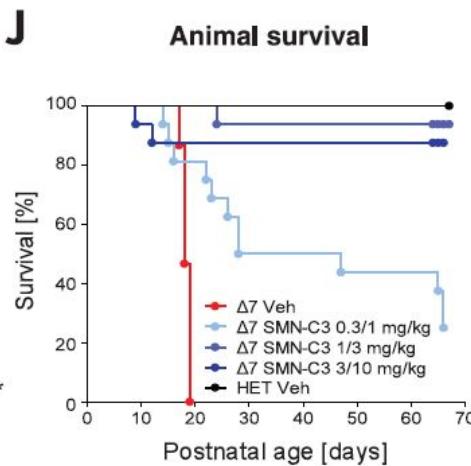
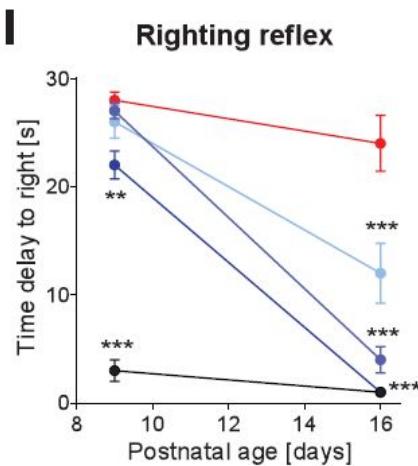
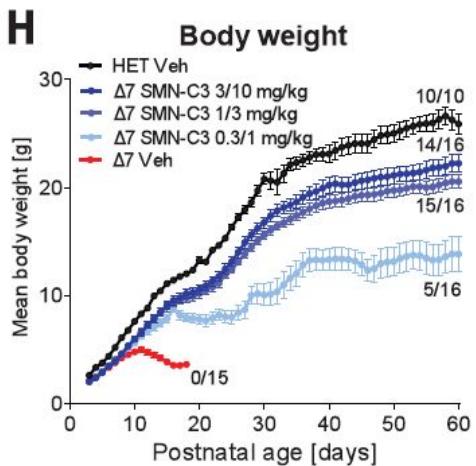
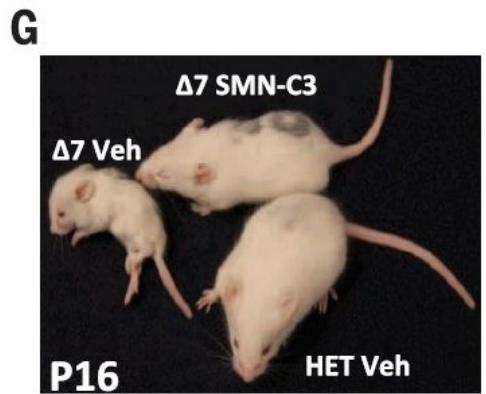
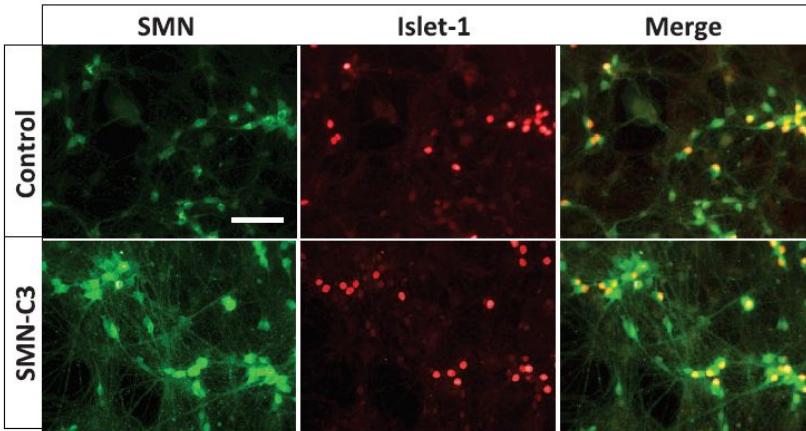


# Gene-enrichment analysis confirms specific regulation of RNA splicing

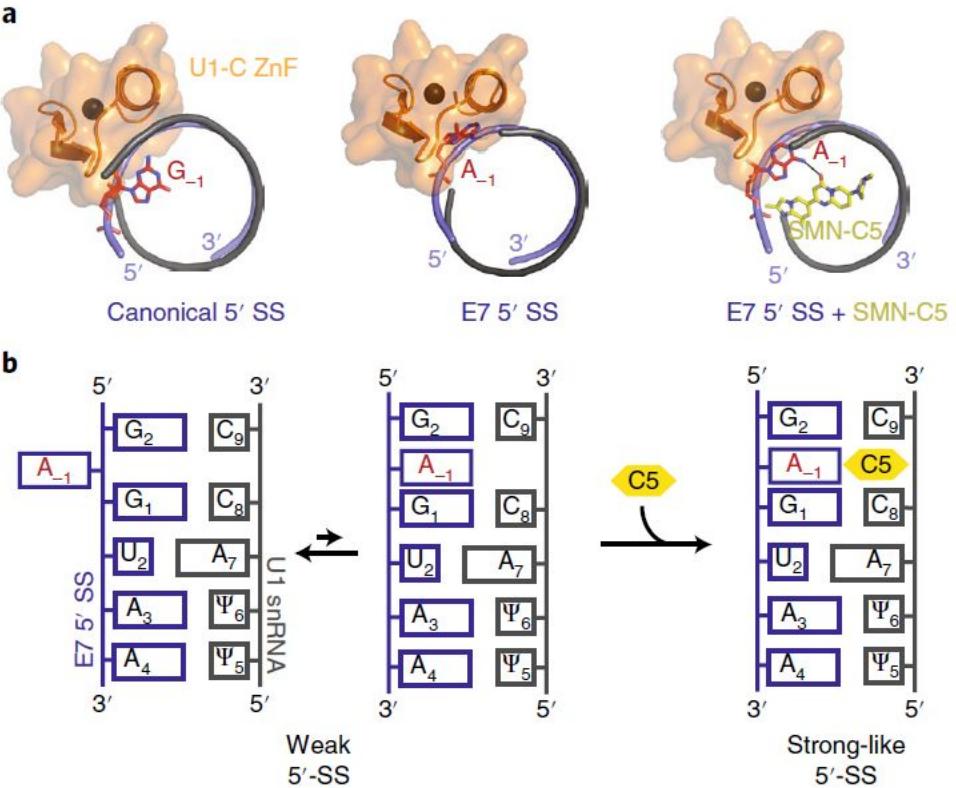
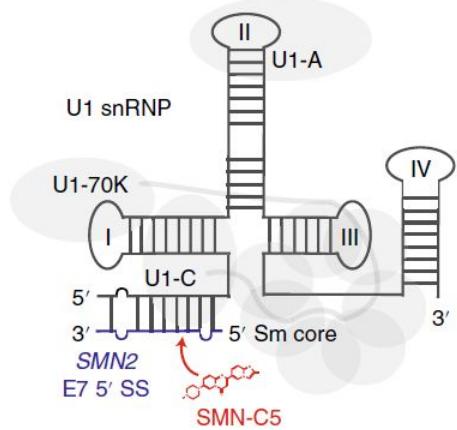
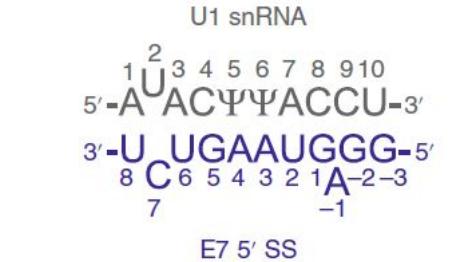


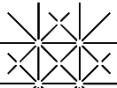
## Part of the mRNA splicing pathway in Reacome

# Experiments *in vitro* and *in vivo* support efficacy profiles of SMN-C3



# Structural basis of specific splicing correction





# Clinical trial ([FIREFISH](#) Part 1) Results

**Table 1.** Demographic and Clinical Characteristics of the Patients at Baseline.\*

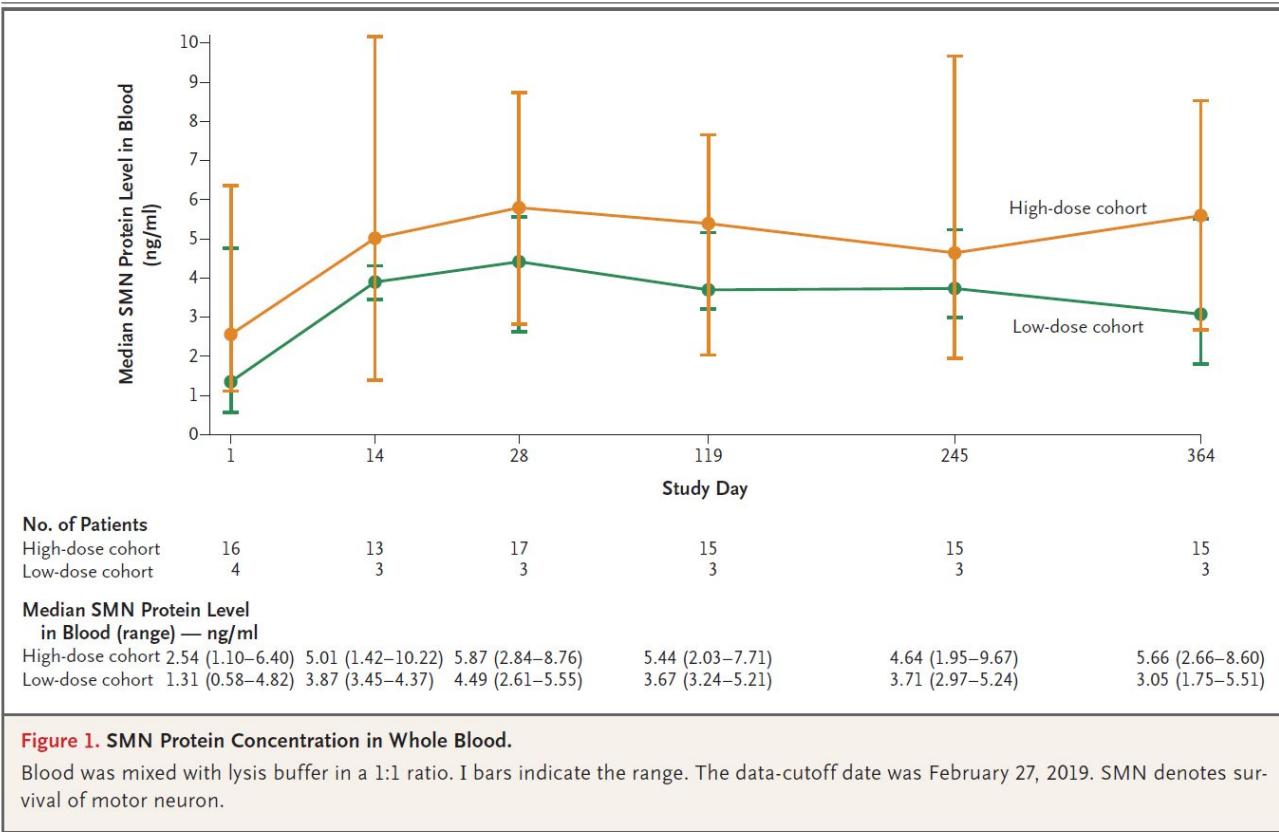
Characteristic	Low-Dose Cohort (N=4)	High-Dose Cohort (N=17)	All Infants (N=21)
Sex — no. (%)			
Female	4 (100)	11 (65)	15 (71)
Male	0	6 (35)	6 (29)
Median age (range) — mo			
At onset of symptoms	2.7 (2.0–3.0)	1.5 (0.9–3.0)	2.0 (0.9–3.0)
At diagnosis	3.3 (2.5–5.1)	3.0 (0.9–5.4)	3.0 (0.9–5.4)
At enrollment	6.9 (6.7–6.9)	6.3 (3.3–6.9)	6.7 (3.3–6.9)
Motor measures†			
Median CHOP-INTEND score (range)	23.5 (10–25)	24 (16–34)	24 (10–34)
Median HINE-2 score (range)	1 (0–3)	1 (0–2)	1 (0–3)
Respiratory support — no. (%)	0	5 (29)‡	5 (24)‡

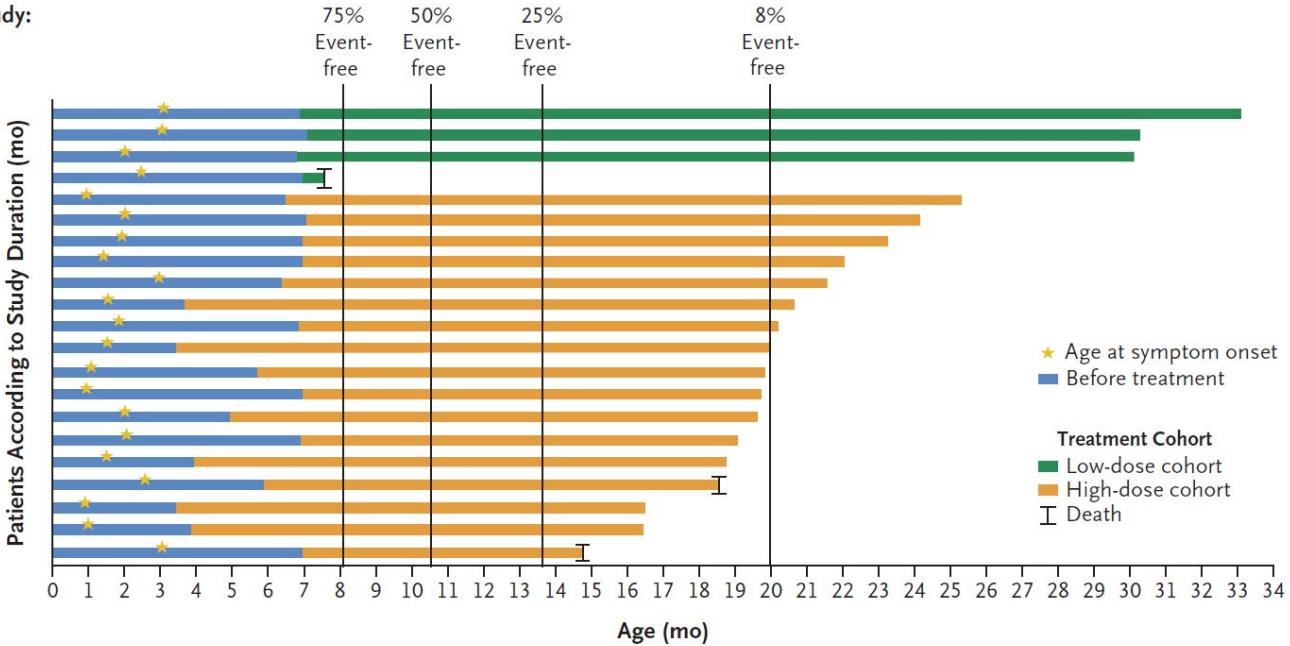
Note: [Table 2](#) is not complete

**Table 2.** Adverse Events.\*

Event	Infants (N=21)
Total no. of adverse events	202
≥1 Adverse event — no. (%)	21 (100)
Total no. of serious adverse events	24
≥1 Serious adverse event — no. (%)	10 (48)
≥1 Adverse event of grade 3–5 — no. (%)	9 (43)
Serious adverse event with fatal outcome — no. (%)†	3 (14)
Most common adverse events — no. (%)‡	
Pyrexia	11 (52)
Upper respiratory tract infection	9 (43)
Diarrhea	6 (29)
Cough	5 (24)

# Clinical trial (FIREFISH Part 1) Results



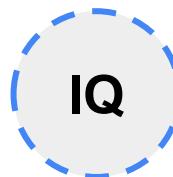
**Natural History Study:**


**Figure 2. Event-free Survival.**

Event-free survival was defined as being alive and not receiving permanent ventilation (tracheostomy or ventilation [bilevel positive airway pressure] for  $\geq 16$  hours per day continuously for  $>3$  weeks or continuous intubation for  $>3$  weeks, in the absence of, or after the resolution of, an acute reversible event). The percentages of patients who were event-free in a previous natural history study of spinal muscular atrophy<sup>7</sup> are shown at the top of the graph for comparison. The median age at the combined outcome among patients in the previous study who had two copies of *SMN2* was 10.5 months (interquartile range, 8.1 to 13.6); event-free survival in that study was defined as being alive and not receiving noninvasive ventilation for 16 hours or more per day continuously for 2 or more weeks. The duration of our study was measured from the date of enrollment to the data-cutoff date. As of the data-cutoff date, three infants (one in the low-dose cohort and two in the high-dose cohort) had died; one additional infant in the high-dose cohort died after that date (Table S5).

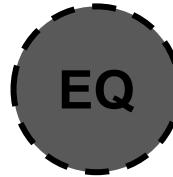
# End of lecture 7 in 2024

# A fallible fairy tale about intelligence



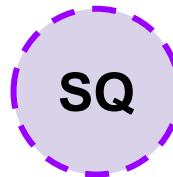
**IQ**

Intellectual  
intelligence



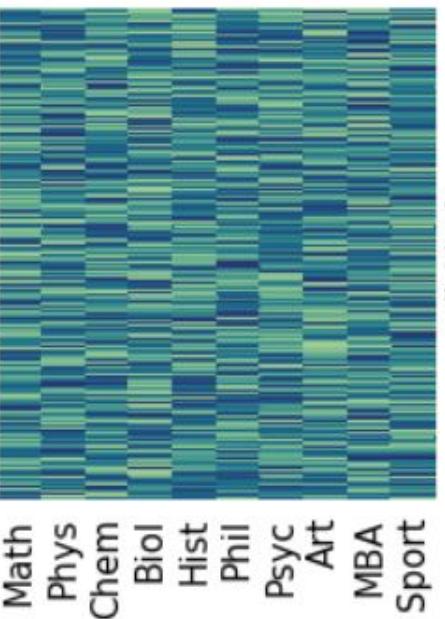
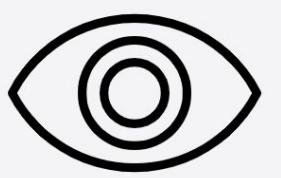
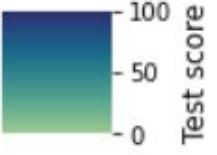
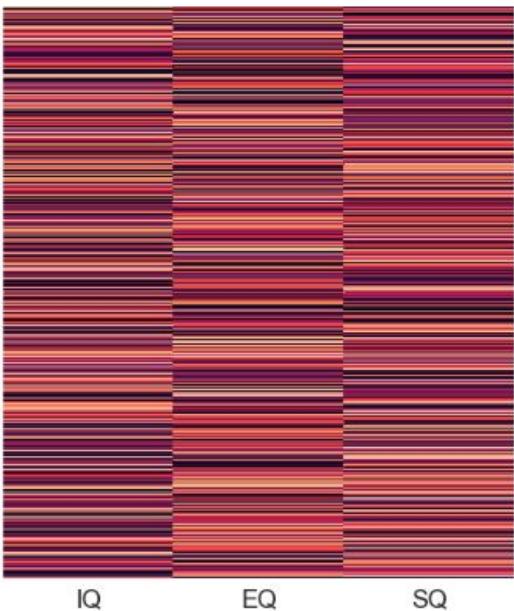
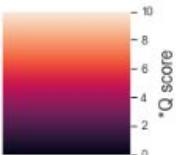
**EQ**

Emotional  
intelligence

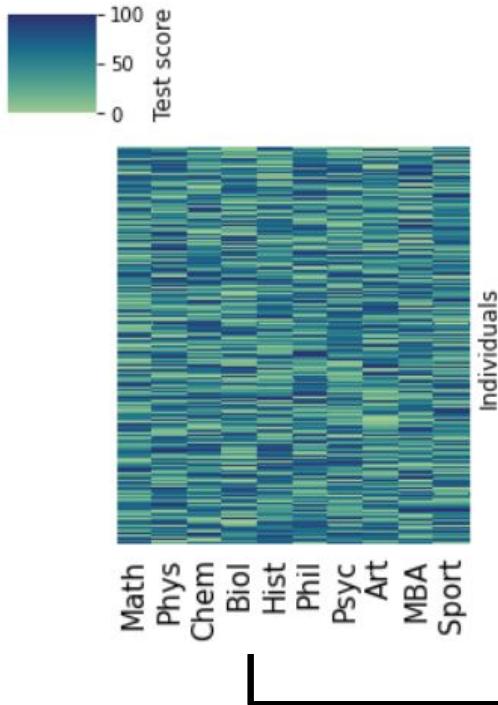


**SQ**

Spiritual  
intelligence



We may infer  $*Q$  if  $*Qs$  are independent and test scores are largely determined by individual  $*Qs$



$$\text{Math} = 8 \times \text{IQ} + 2 \times \text{EQ} + 2 \times \text{SQ} + \varepsilon$$

$$\text{Philosophy} = 6 \times \text{IQ} + 2 \times \text{EQ} + 8 \times \text{SQ} + \varepsilon$$

...

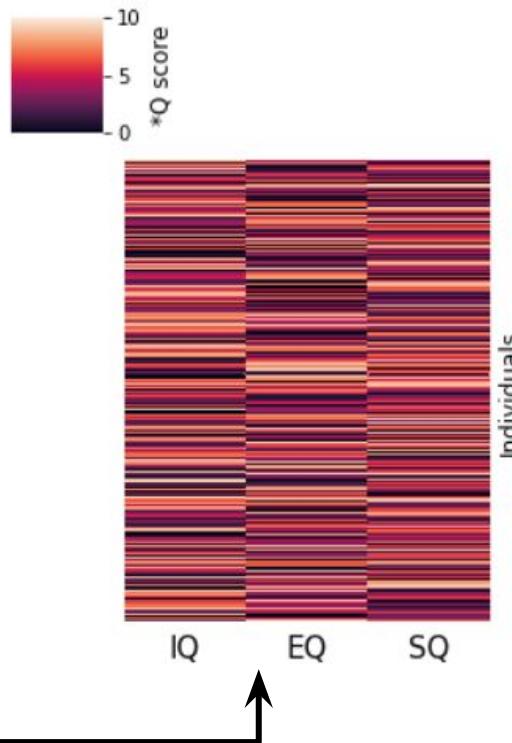
$$\text{MBA} = 4 \times \text{IQ} + 7 \times \text{EQ} + 5 \times \text{SQ} + \varepsilon$$

$$\text{Sport} = 5 \times \text{IQ} + 6 \times \text{EQ} + 5 \times \text{SQ} + \varepsilon$$

**Numbers in blue:** loadings

**IQ/EQ/SQ:** factors

Factor analysis

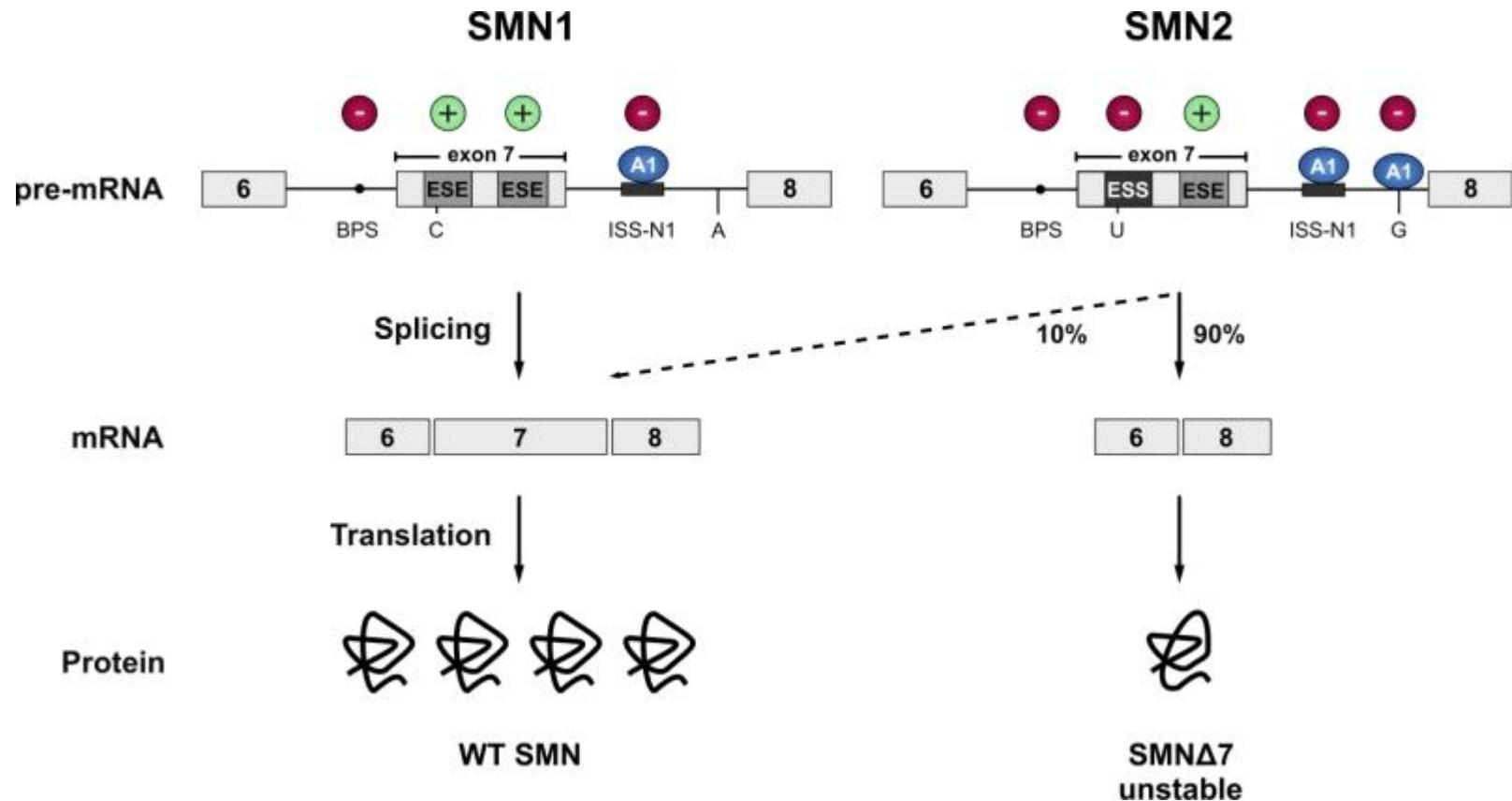


# Offline Activities for Module III

Use your favorite literature programming tools (i.e. Rmarkdown/Jupyter Notebook) to investigate the topic of *factor analysis*. Use the questions below to guide your learning.

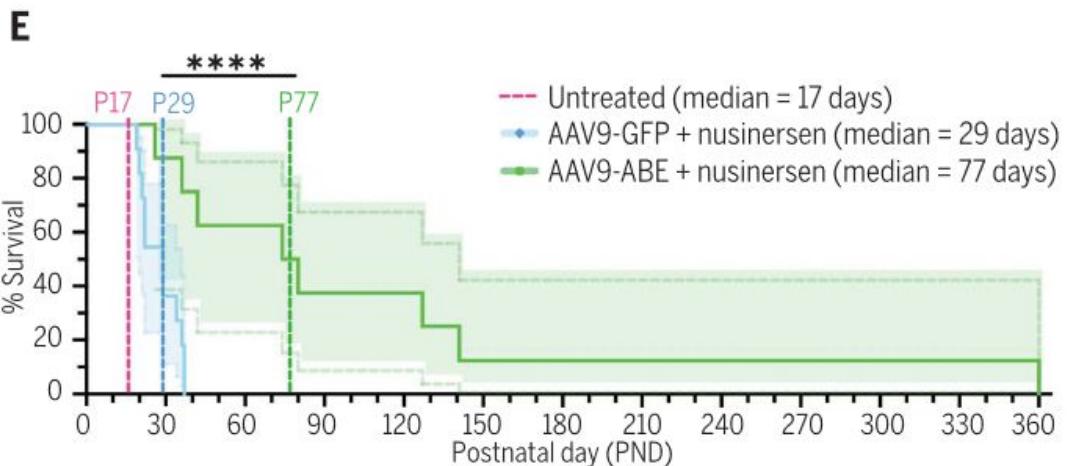
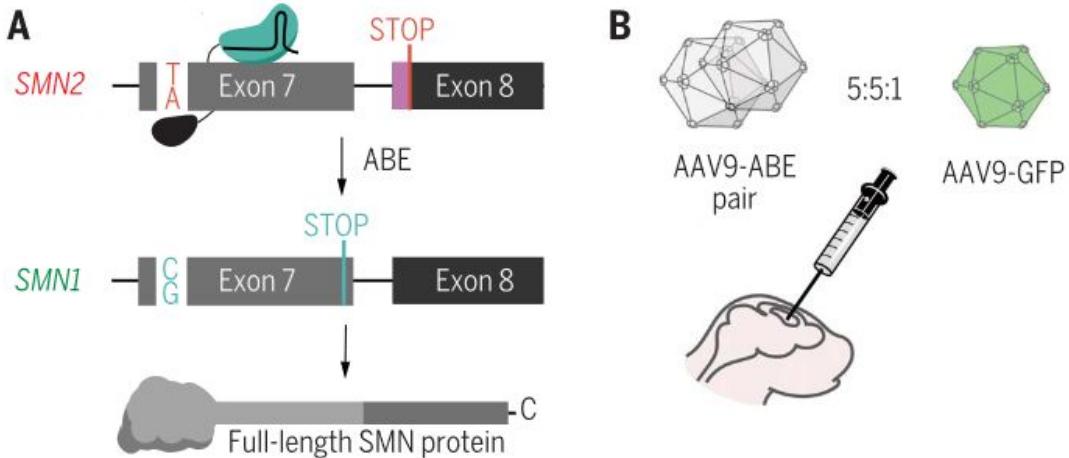
- What is factor analysis?
- What are the relationships between covariance matrix, factor analysis, and principal component analysis (PCA)?
- What do we mean with *loadings*?
- Why factors are orthogonal to each other? What's the consequence?
- How can we use factor analysis as a generative model?
- What is the relationship between factor analysis and autoencoder?
- How can you it explain it to a high-school student?

# Different splicing of SMN1 and SMN2



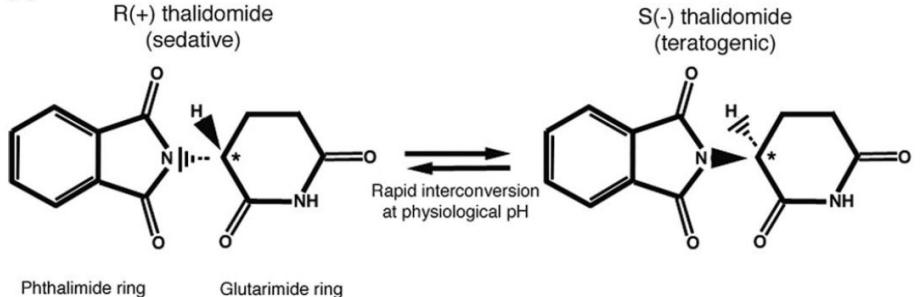
# Base editing rescue of spinal muscular atrophy in cells and in mice

Arbab *et al.*, Science,  
April 2023

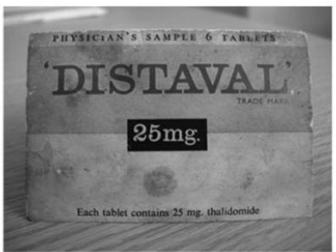


# The Tragedy of teratogenic S(-) thalidomide in 1950s

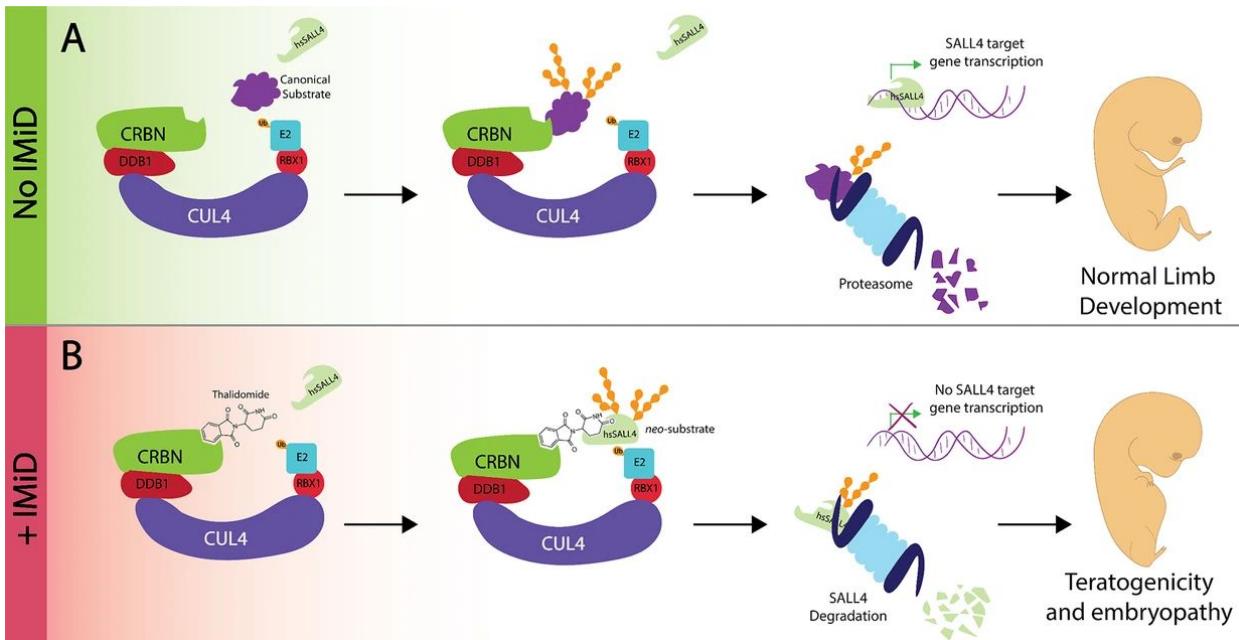
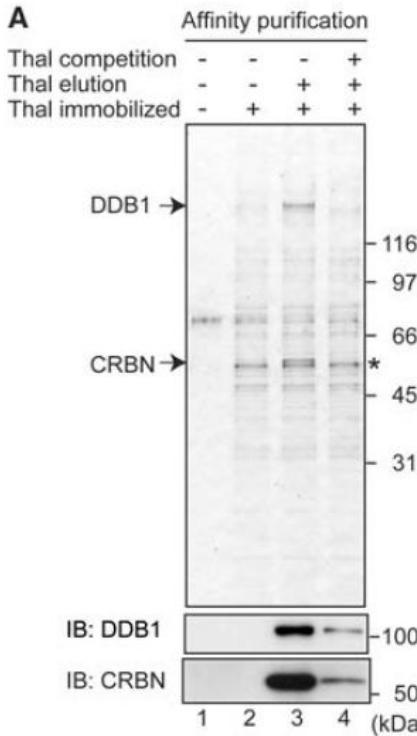
A



B

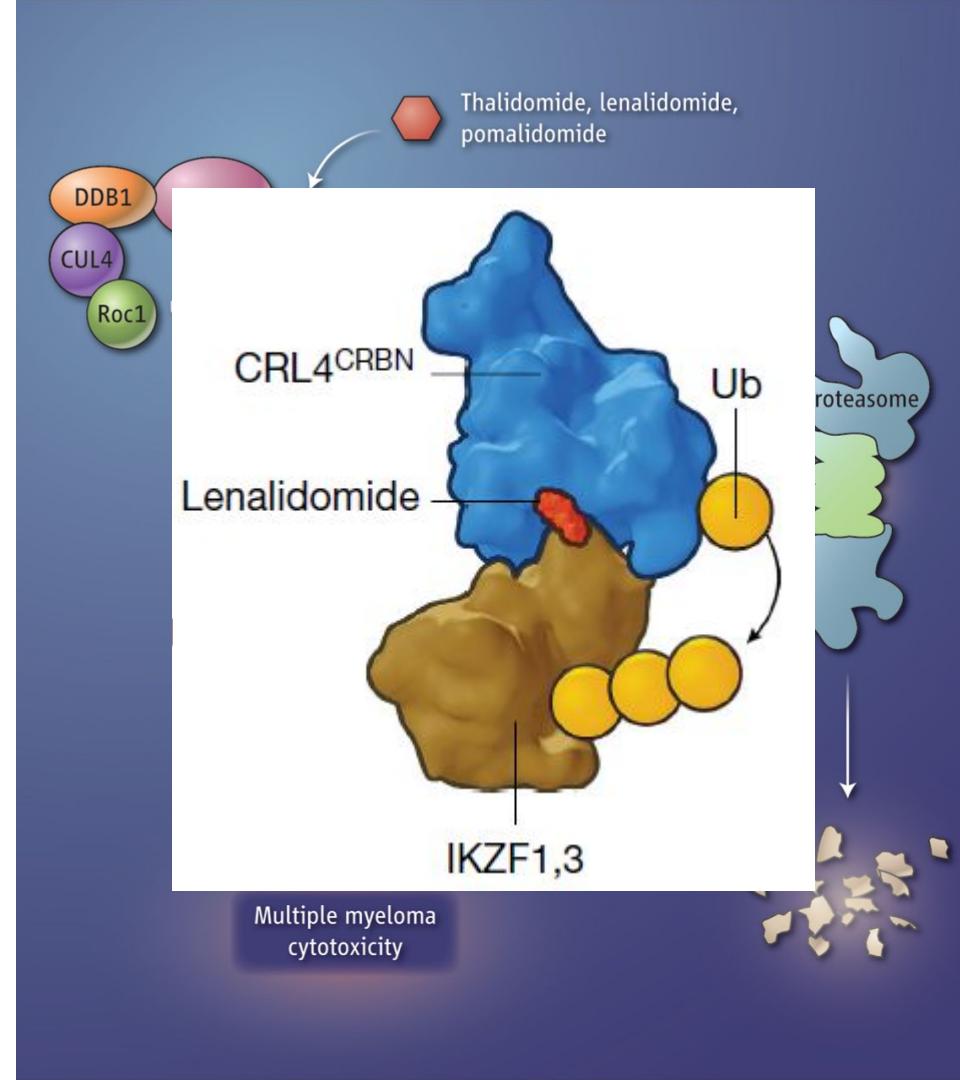


# Molecular basis of the teratogenicity of thalidomide reported in 2010



# The same mechanism is responsible for efficacy against blood cancers

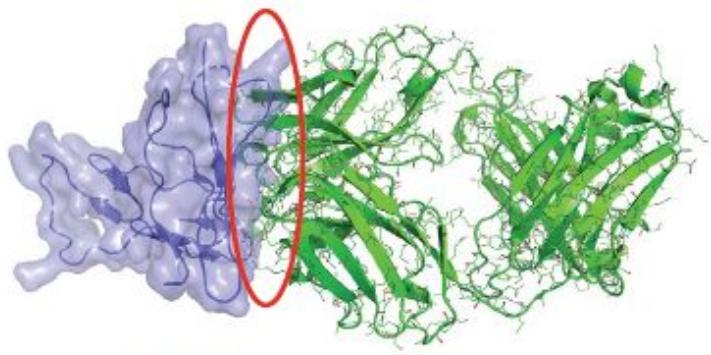
Thalidomide and derivatives bring proteins IKZF1 and IKZF3 close to E3 ubiquitin ligase, leading them to be degraded.



# Multispecific Drug Use or Target Interactions

**b** Conventional drug:

- Forms 1 drug–target interface
- Can act throughout body
- Only works if its binding to target alters function of target



IL-2R $\alpha$

Basiliximab

**c** Obligate multispecific drug:

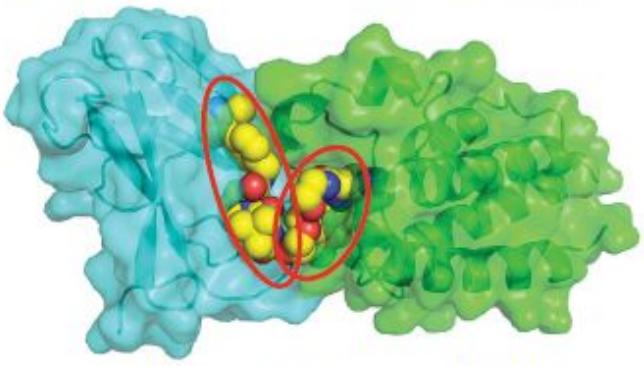
- Forms 2 or more drug–target interfaces

Class 1 ‘tetherbodies’

- Enrich drug at relevant site of action

Class 2 ‘matchmakers’

- Link drug to a biological effector

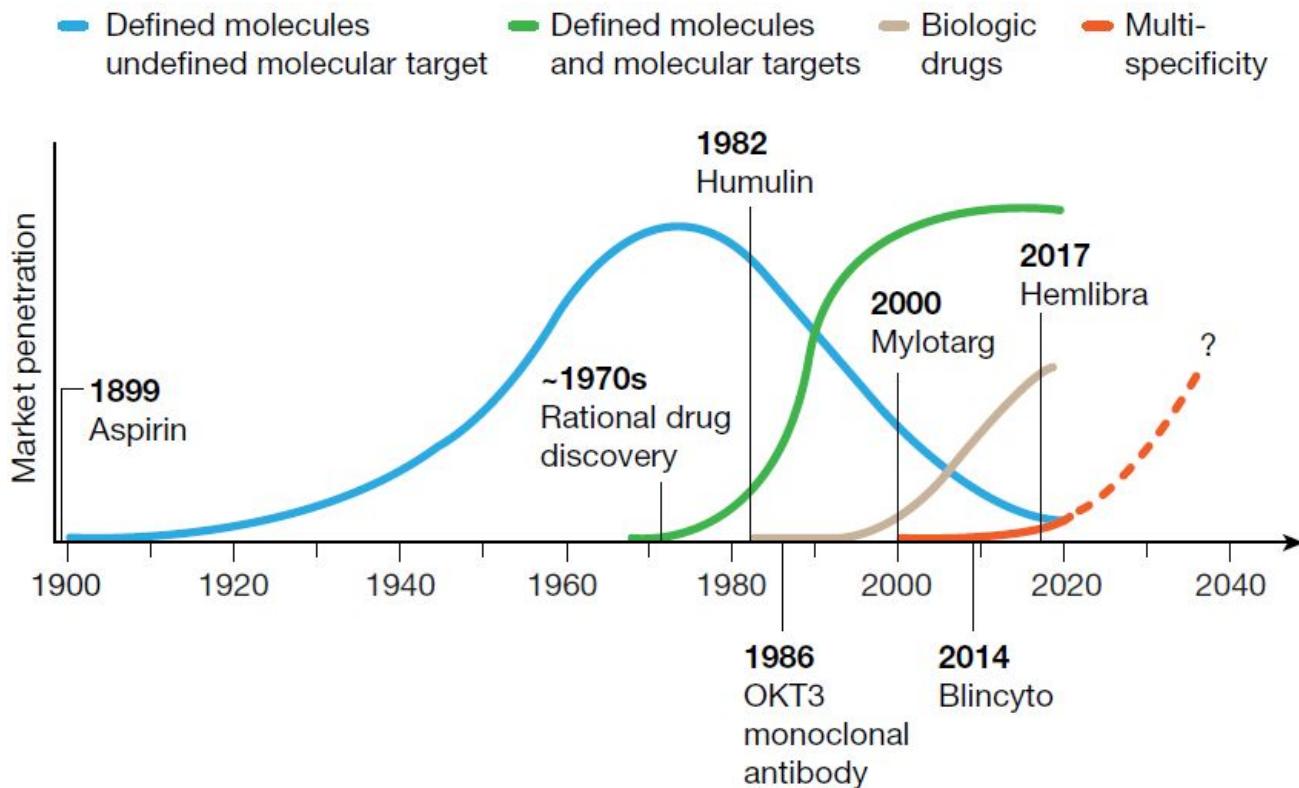


VHL

MZ1

BRD4 $^{BD2}$

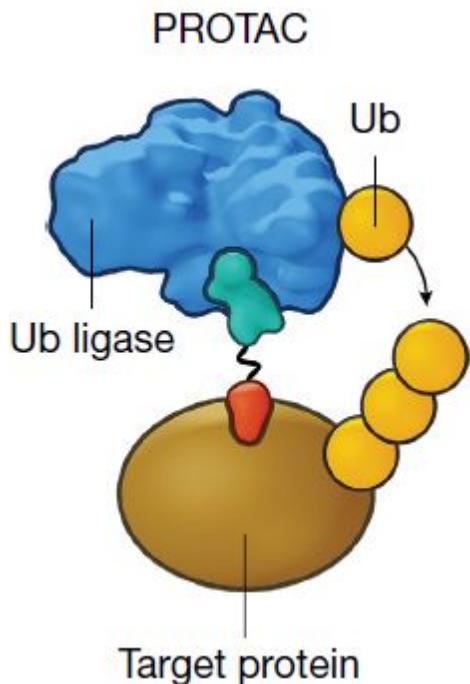
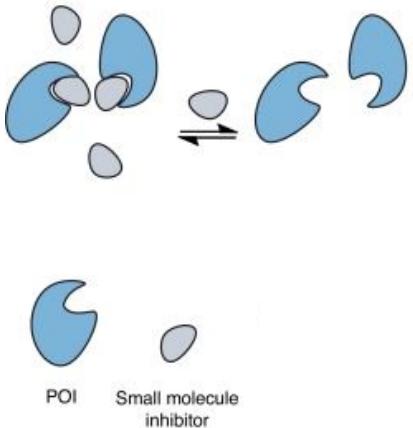
# Paradigm shifts and paradigm expansion



# PROteolysis TArgeting Chimera (PROTAC)

## (a) Occupancy-driven pharmacology

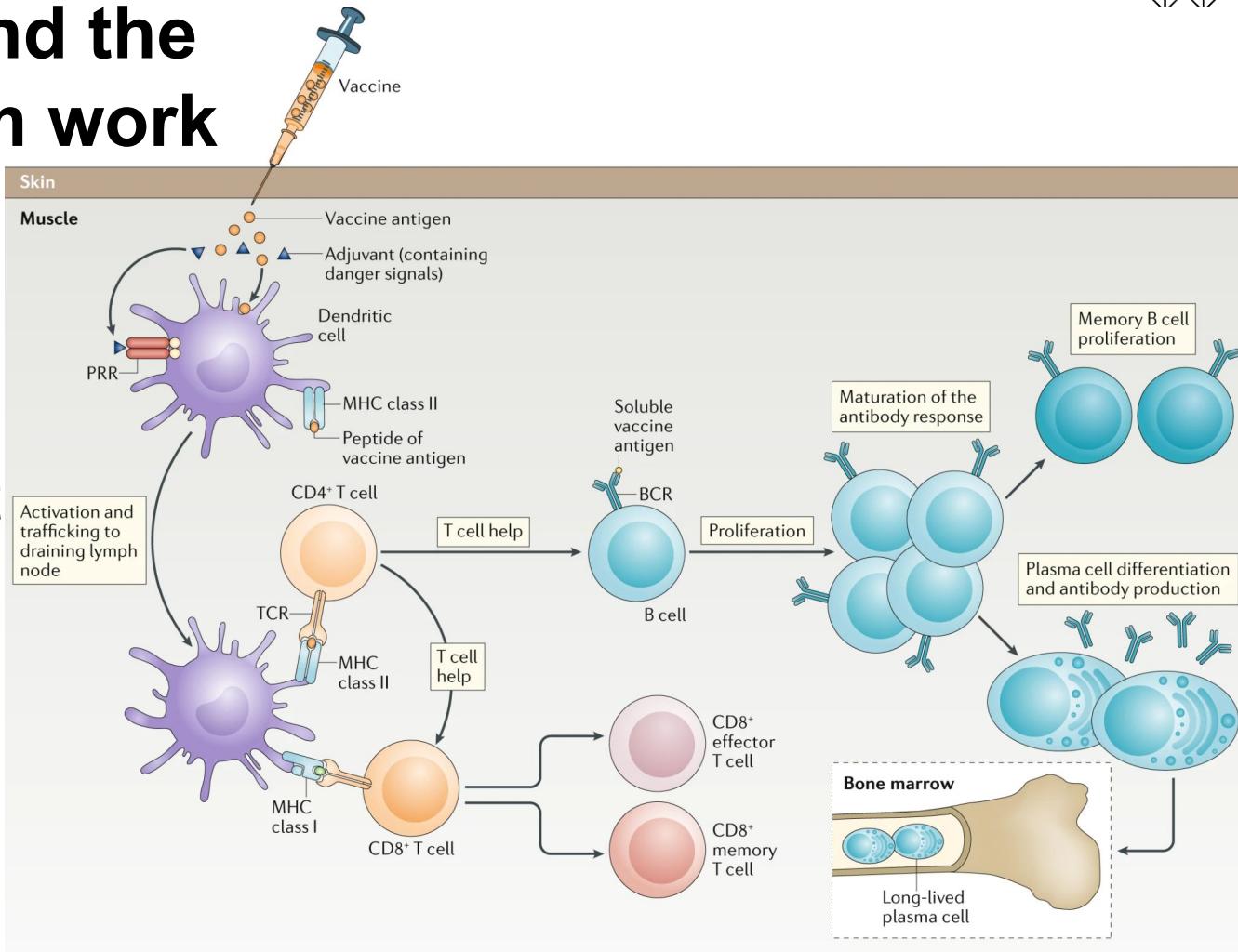
Protein function is modulated *via* inhibition

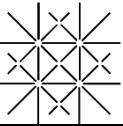


# How vaccine and the immune system work

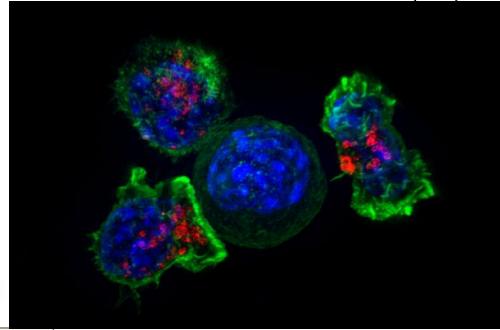
Key players:

1. Antigen-presenting cells (e.g. dendritic cells)
2. T cells
3. B cells

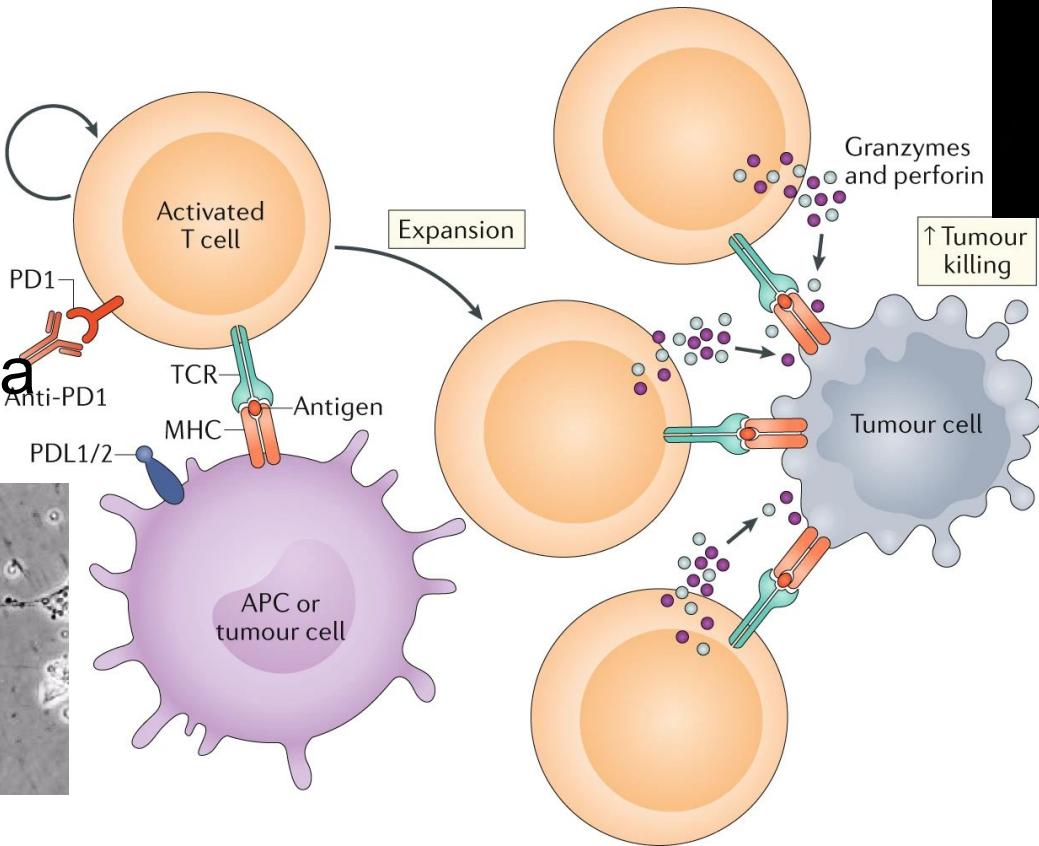
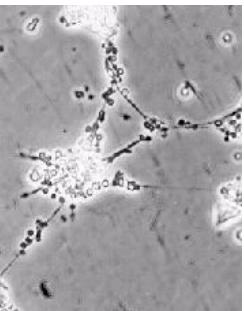




# Antigen-presenting cells (APC) and T cells work together to kill tumour cells

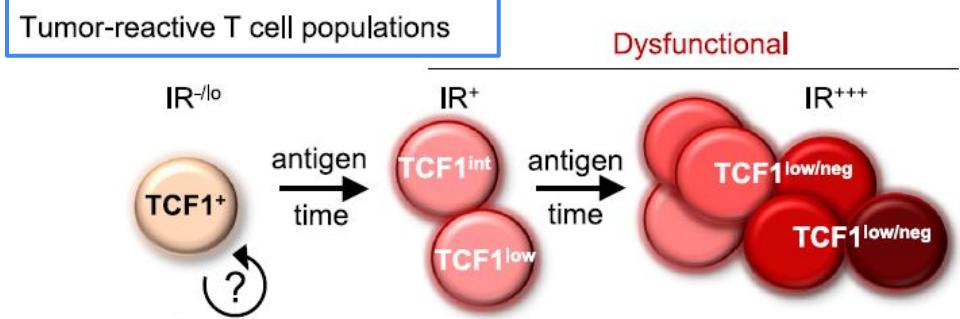
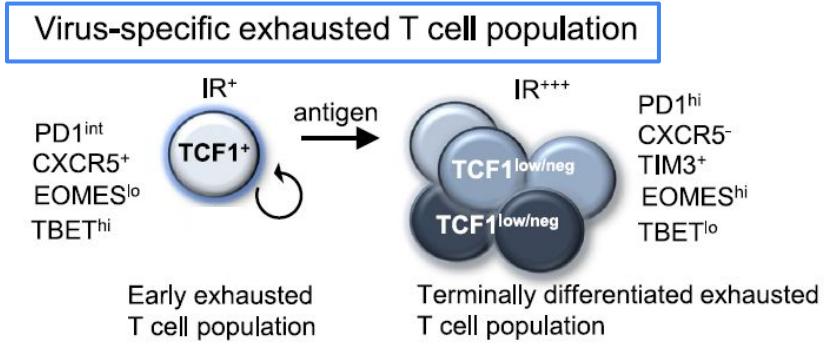
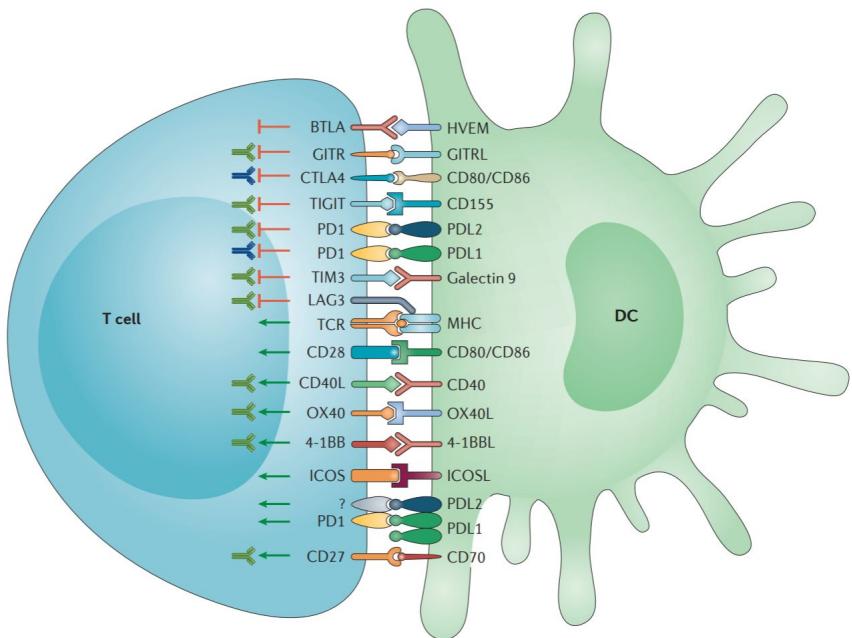


Dendritic cell, a type of APC



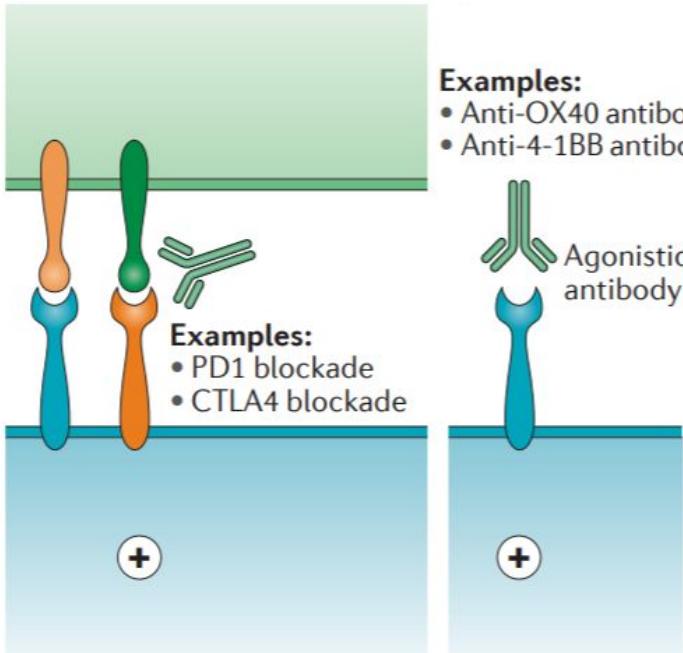
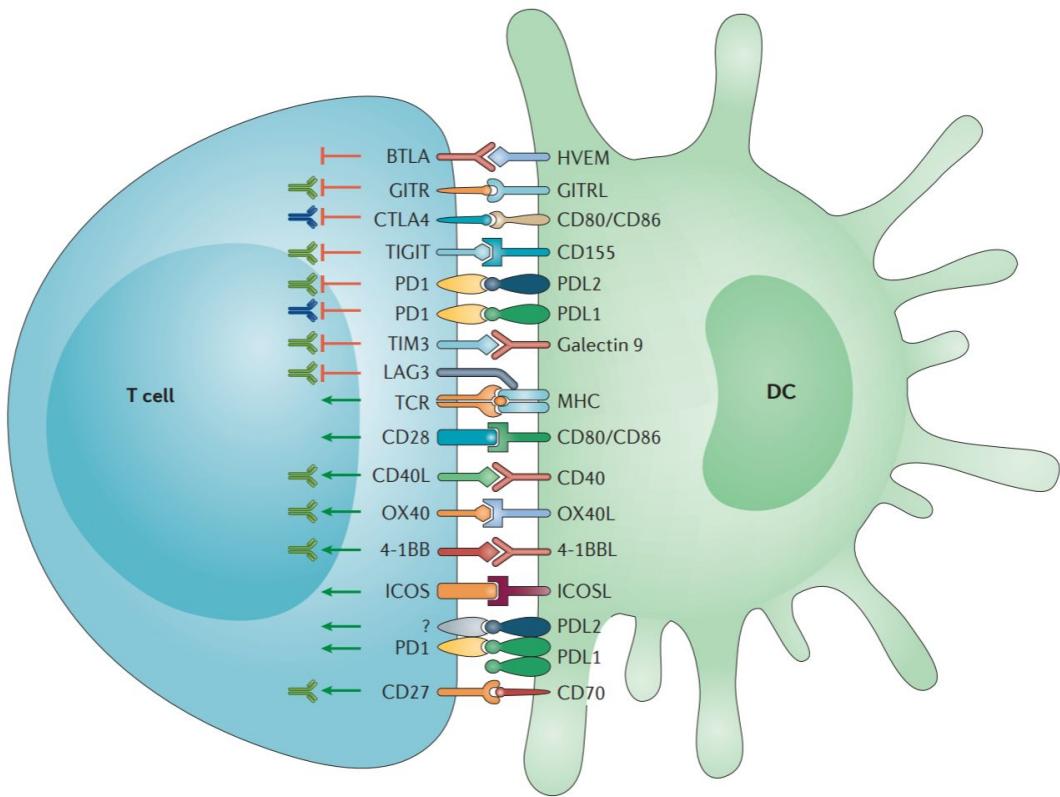
Killer T cells attacking a cancer cell

# Exhausted T cells reduces immune system's capacity to clear pathogenic cells

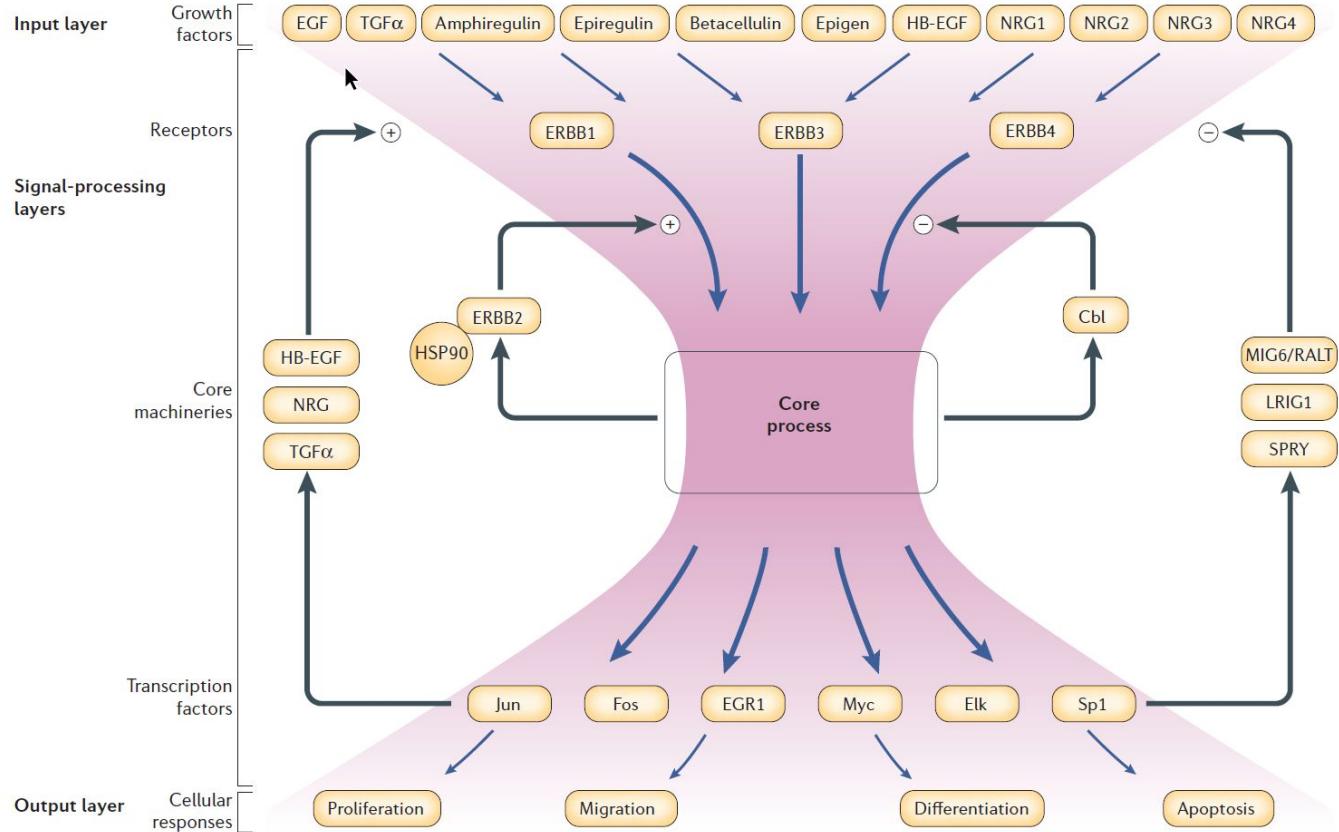


IR=inhibitory receptors (left panel). They are like 'breaks' controlled by dendritic cells.

# Cancer Immunotherapy with immune checkpoints as drug targets



# Why antibodies work like a wonder? The Bow-Tie model of signaling transduction

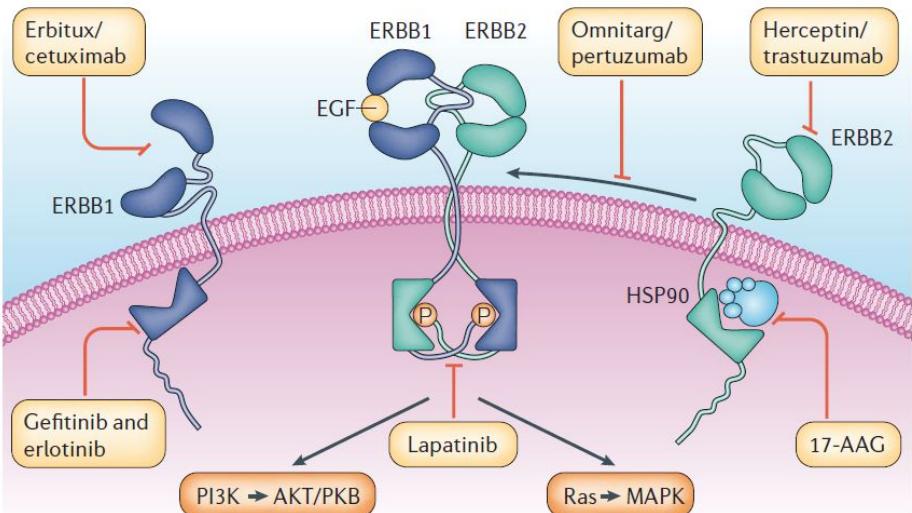
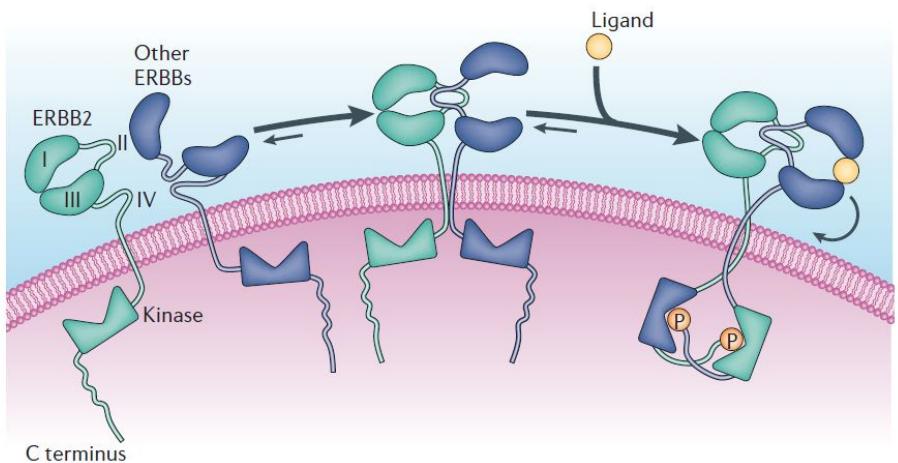


Extracellular  
Cell  
Membrane

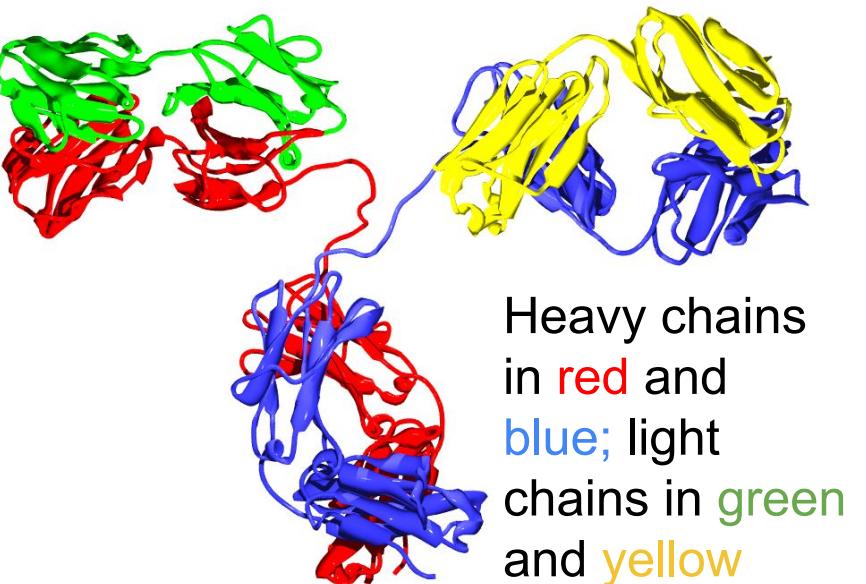
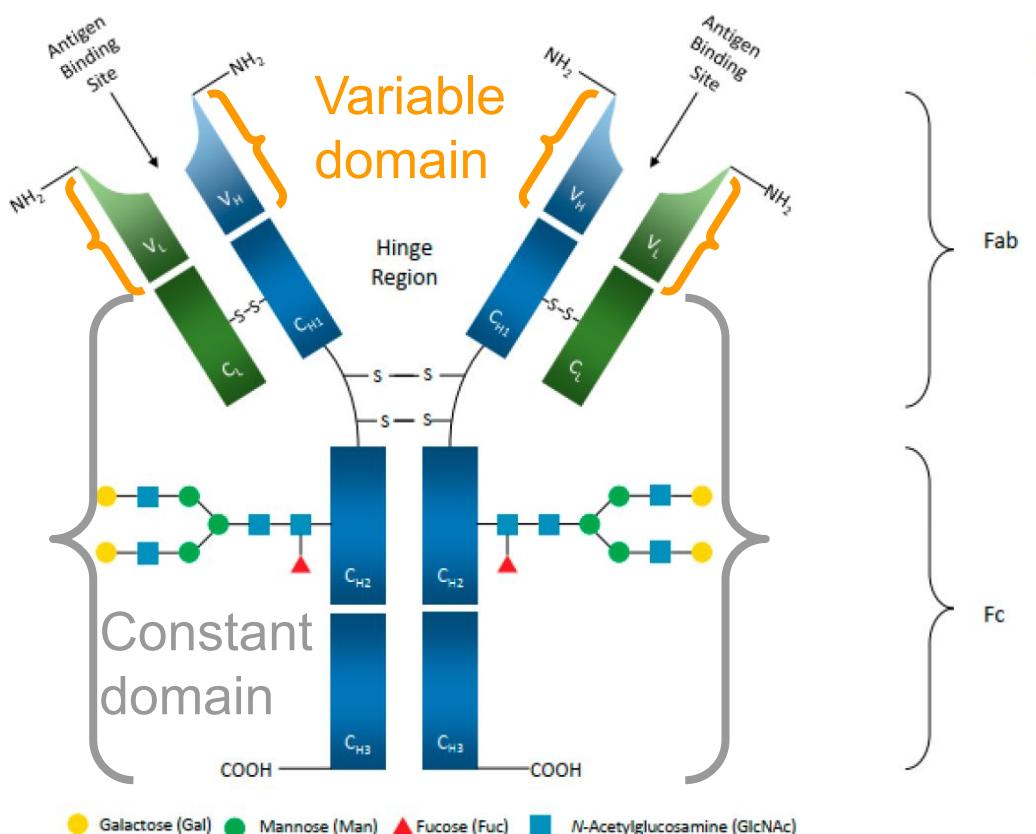
Cytoplasm

Nucleus  
Everywhere

# ERBB signaling system and antibody drugs



# Structure of antibodies



Fc=fragment crystallizable region

Fab=fragment antigen binding

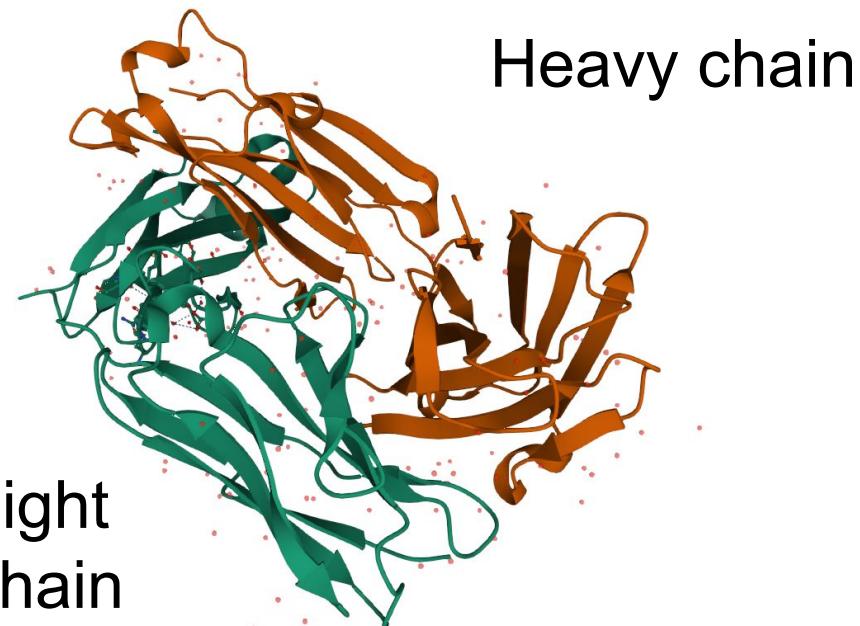
# Cetuximab as an example

## Variable heavy chain

QVQLKQSGPGLVQPSQSL SITCTVSGF  
SLTNYGVHWVRQSPGKGLEWLGVIVSG  
GNTDYNTPFTSRLSINKDNSKSQVFFK  
MNSLQSNDTAIYYCARALTYYDYEFA  
WGQGTLVTVSA

## Variable light chain

DILLTQSPVILSVSPGERVS FSCRASQ  
SIGTNIHWYQQRTNGSPRLLIKYASES  
ISGIPSRFSGSGSGTDFTLSINSVESE  
DIADYYCQQNNNWPTTFGAGTKLELK

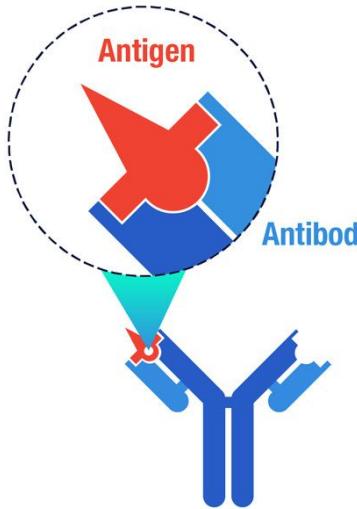


PDB 1YY8

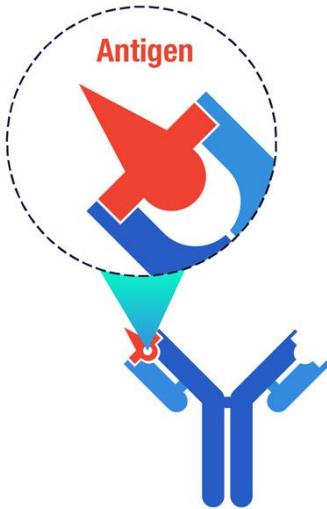
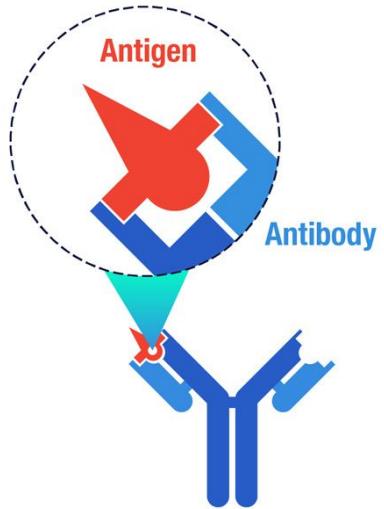
# Antibodies work by shape complementarity

Affinity of antibodies for antigens can vary

High-Affinity Binding

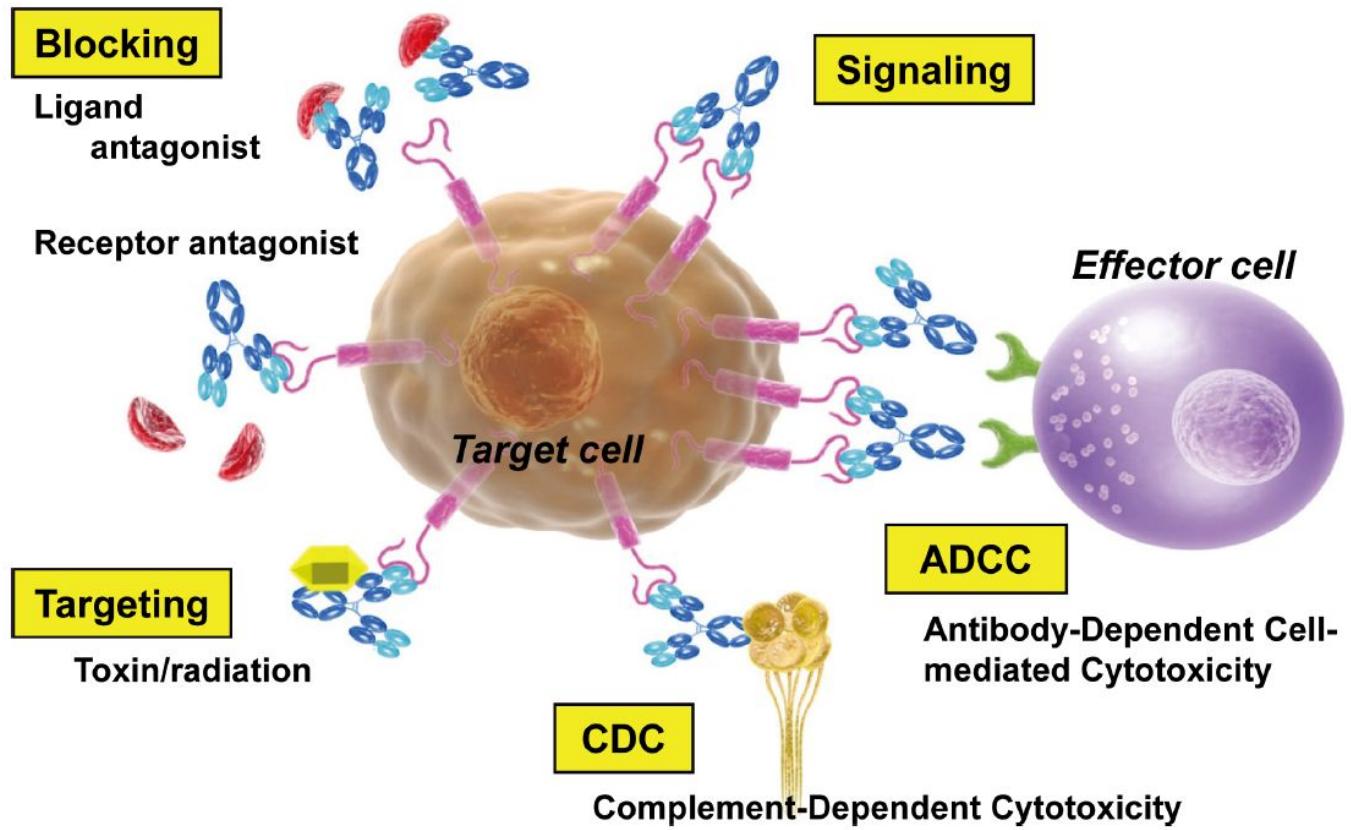


Low-Affinity Binding

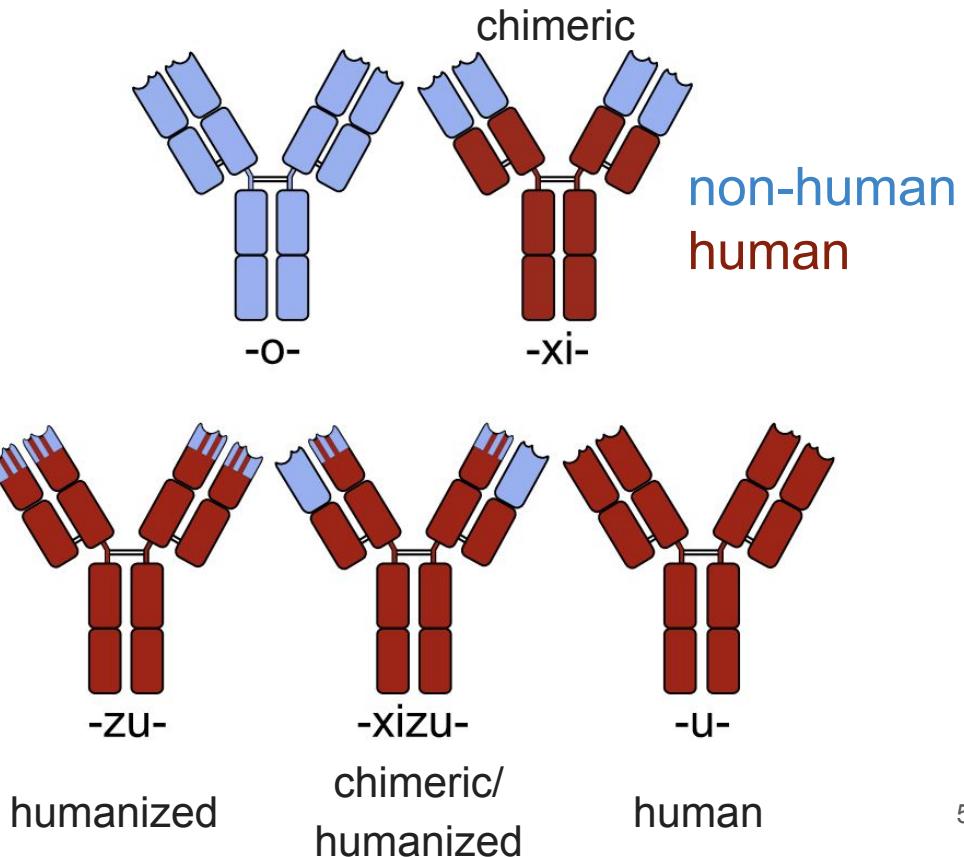
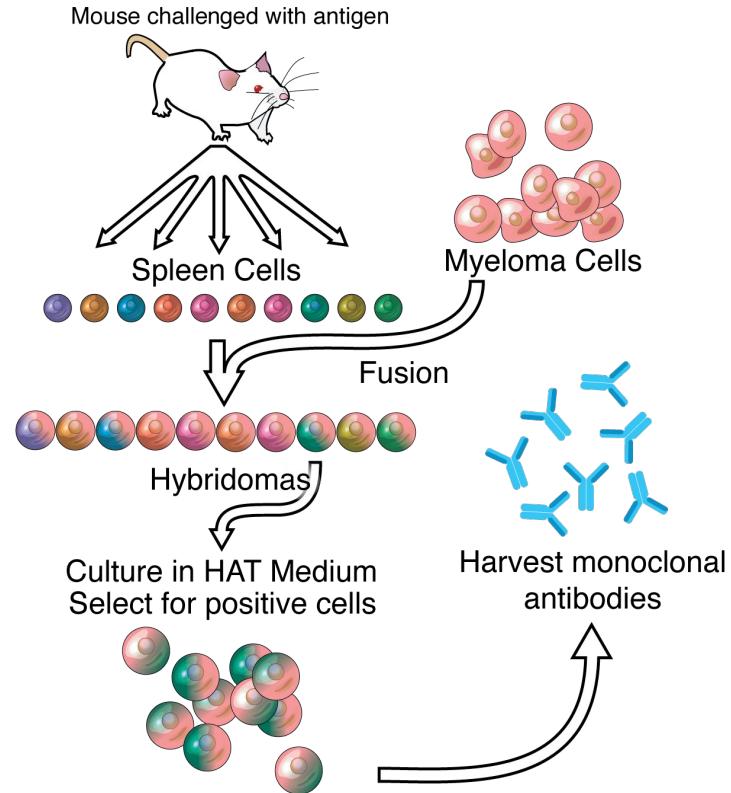


Recommended reading: [10 things to know about antibodies](#) by Amgen

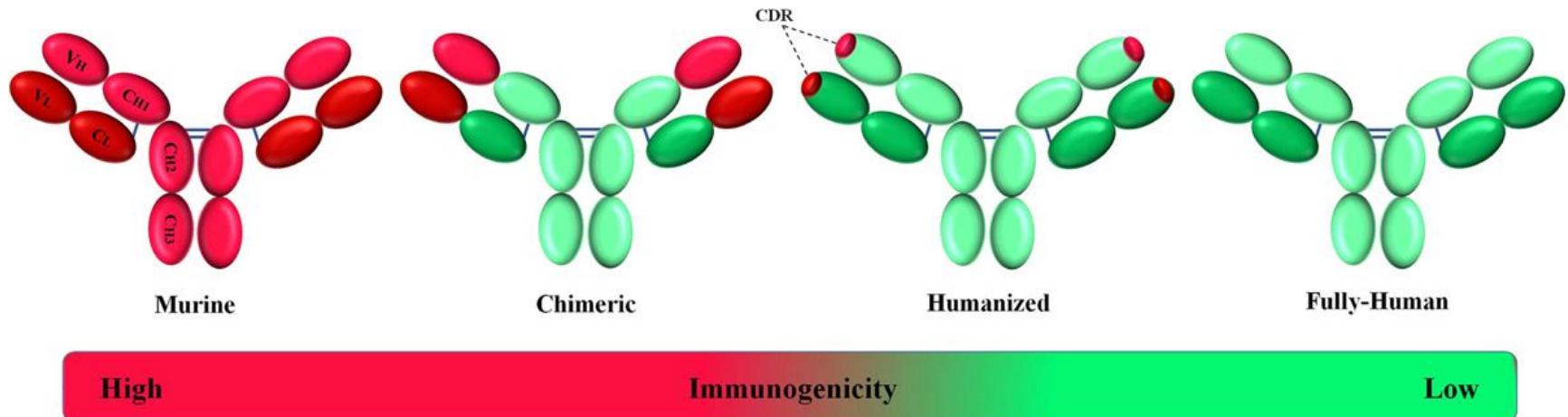
# Mechanisms of action of therapeutic antibodies



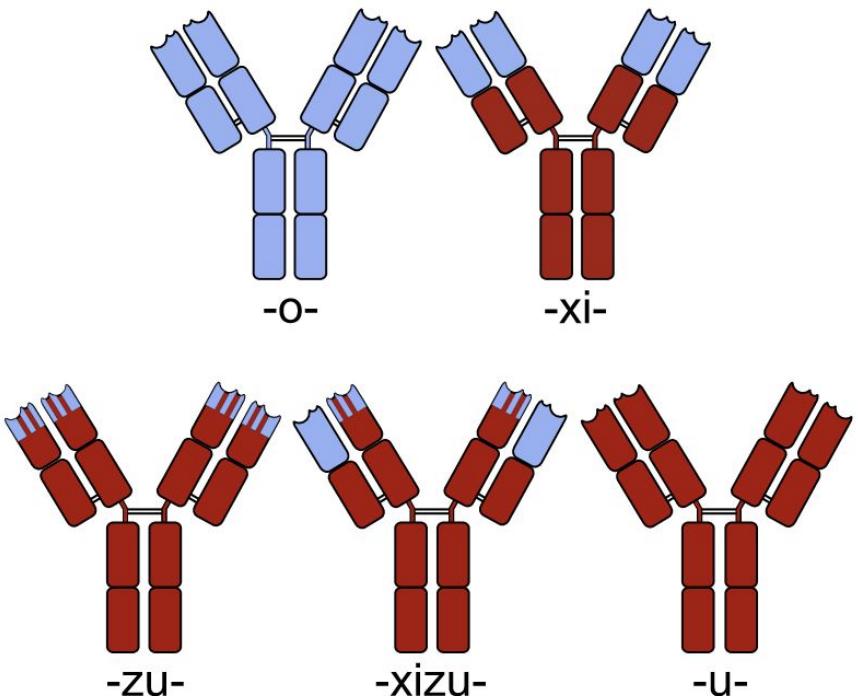
# Therapeutic antibody discovery with hybridoma and humanization



# Evolution of therapeutic antibodies



# Antibody names suggest their types

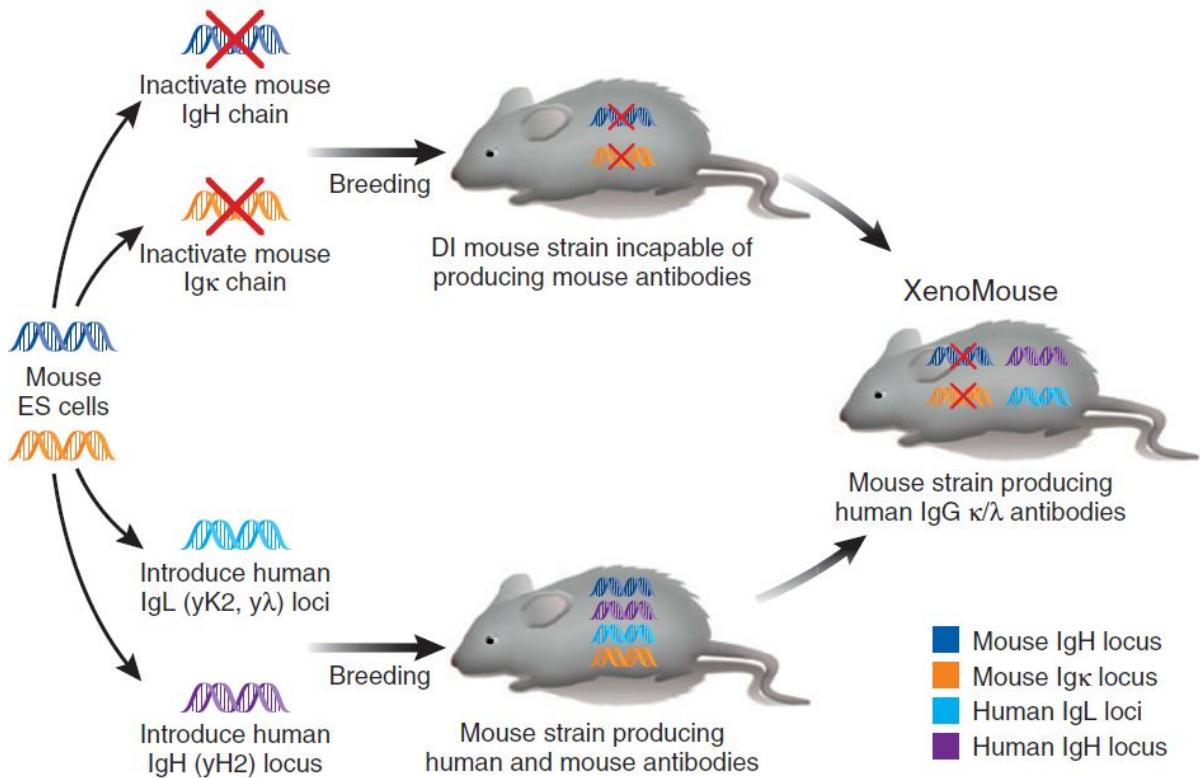


- **Chimeric:** Abiciximab (Ab against platelet aggregation inhibitor)
- **Humanized:** Trastuzumab (HER2)
- **Chimeric/Humanized:** Otelixizumab (CD3, a T lymphocyte receptor)
- **Human:** Adalimumab (TNF-alpha)

# Therapeutic antibody discovery with transgenic animals

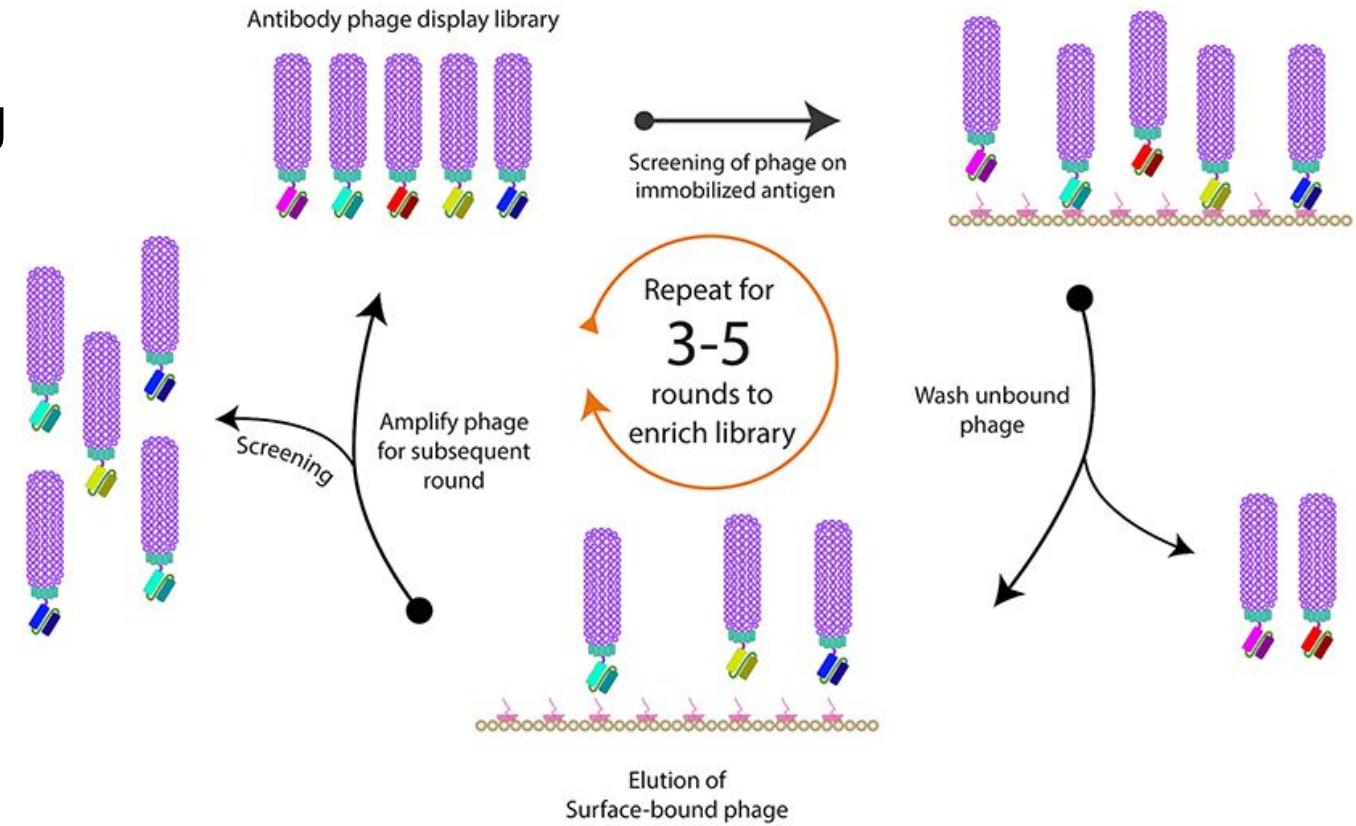
The XenoMouse model, which led to the discovery of panitumumab (Vectibix).

Panitumumab targets EGFR for advanced colorectal cancer.



# The principle of phage display

A protein-encoding gene is inserted into the phage coat protein gene, causing the phage to **display** the protein, which can be screened *in vitro* iteratively.



# Antibody discovery with phage display

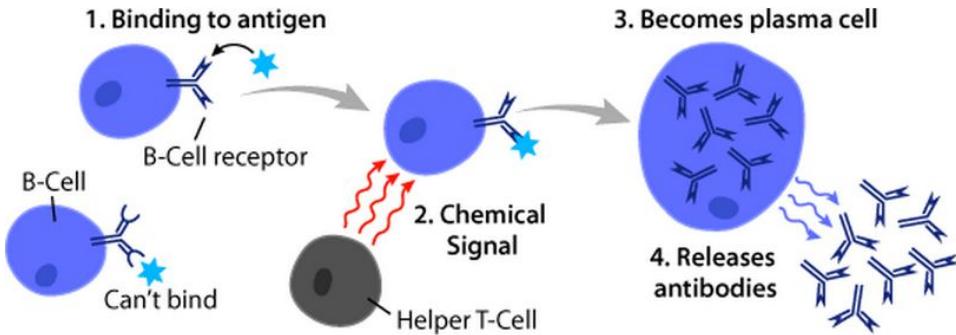
Convalescent or patient blood plasma

RNA → cDNA

Copy antibody-coding sequences

Paste sequences into phage coats

Copy/Paste: PCR and cloning

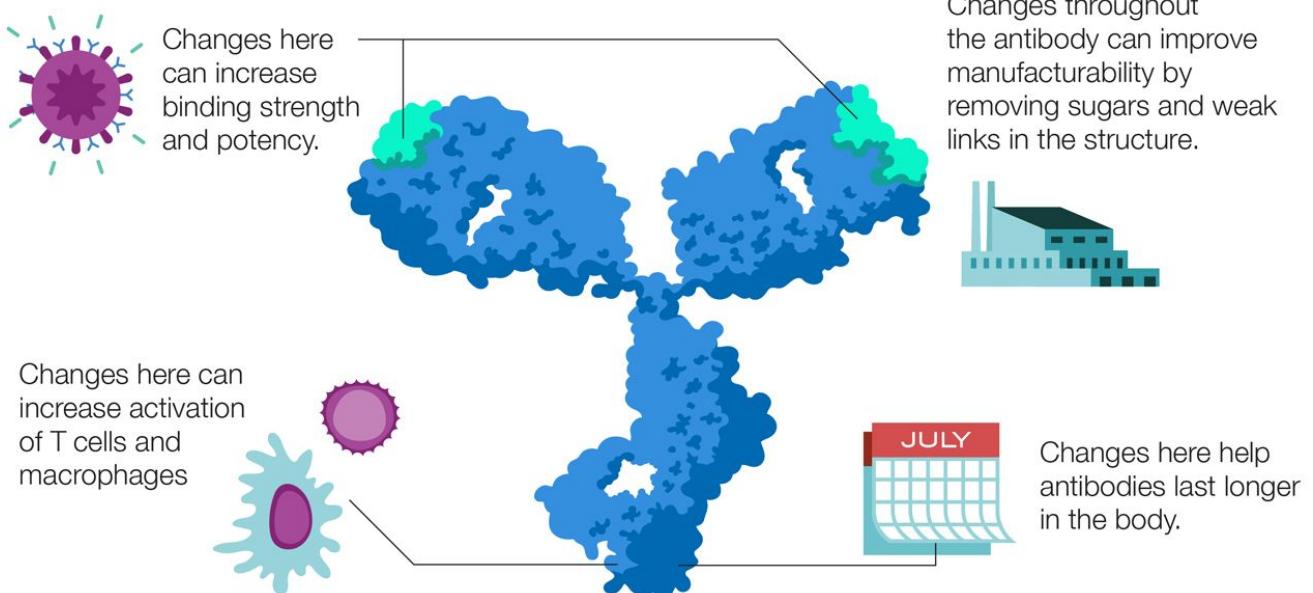


**Aim:** Reverse-engineering existing antibodies

Antibody selection

# Discovered antibodies need further development

## Engineering antibodies with strong attributes



# Major challenges of antibody discovery and development

- *Lack of quantitative rules of developability*
- Immunogenicity of therapeutic proteins (see backup)

# Biophysical properties of clinical-stage antibodies (N=137 by ~2017)

Name	Light chain class	Type	Original mAb Isotype or Format	Clinical Status	Phage <sup>c</sup>	Year Name Proposed				
			IgG2	Phase 2	No.	2013				
abituzumab	kappa	ZLL	IgG2	Phase 2	No.	2013				
abrilumab	kappa	H	Name	VH	VL	LC Class	Source	Source Detailed <sup>a</sup>		
adalimumab	kappa	H	abituzumab	QVQLQQSGGELAKPGASVKVSCKASGDIQMTQS	kappa	WHO-INN	PL109			
			abrilumab	QVQLVQSGAEVKPGASVKVSCKVSGDIQMTQS	kappa	WHO-INN	PL111			
alemtuzumab	kappa	H	adalimumab	EVQLVESGGGLVQPGRSLRLSCAASGFDIQMTQS	kappa	PDB	4NYL			
alirocumab	kappa	H	alemtuzumab	QVQLQESGPGLVRPSQTLSTLCTVSGFDIQMTQS	kappa	PDB	1BEY			
anifrolumab	kappa	H	alirocumab	EVOI VESGGGI VOPGGSLRISCAASGDIVMTOSI	kappa	WHO-INN	PI 107			

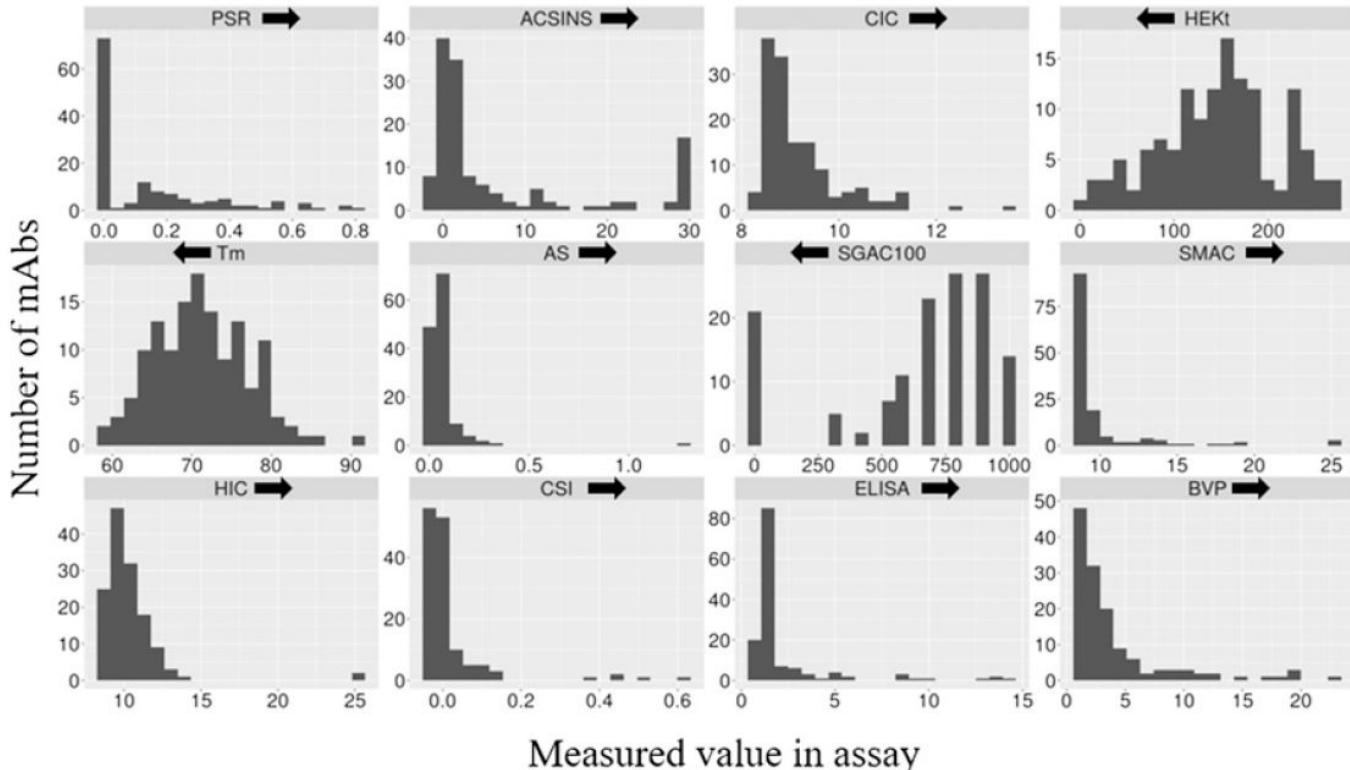
Name	HEK Titer (mg/L)	Fab Tm by DSF (°C)	SGAC-SINS AS100 ((NH4)2SO4 mM)	HIC Retention Time (Min) <sup>a</sup>	SMAC Retention Time (Min) <sup>a</sup>	Slope for Accelerated Stability	Poly-Specificity Reagent (PSR) SMP Score (0-1)	Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS) Δλmax (nm) Average	CIC Retention Time (Min)	CSI-BLI Delta Response (nm)	ELISA	BVP ELISA
								Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS) Δλmax (nm) Average				
abituzumab	89.6	75.5	900.0	9.2	8.7	0.06	0.17	1.5	8.6	0.00	1.14	2.72
abrilumab	100.2	71.0	900.0	9.4	8.7	0.03	0.00	-0.9	8.4	-0.02	1.12	1.82
adalimumab	134.9	71.0	900.0	8.8	8.7	0.05	0.00	1.1	8.9	-0.01	1.08	1.49
alemtuzumab	144.7	74.5	1000.0	8.8	8.7	0.06	0.00	-0.8	8.5	-0.02	1.16	1.46
alirocumab	69.2	71.5	900.0	9.0	8.7	0.03	0.00	1.2	8.8	-0.01	1.20	2.18
anifrolumab	82.0	62.5	700.0	8.8	8.6	0.07	0.00	-0.6	8.5	-0.02	1.16	1.62
atezolizumab	164.1	73.5	300.0	13.4	19.3	0.06	0.07	15.0	10.8	0.06	1.29	6.20
bapineuzumab	151.1	73.0	1000.0	8.9	8.7	0.07	0.00	-0.7	8.6	0.06	1.21	3.55
basiliximab	107.5	60.5	0.0	9.6	8.6	0.05	0.40	28.8	9.4	0.00	1.20	2.14
bavituximab	45.1	59.5	0.0	11.5	12.7	0.04	0.56	29.9	11.4	-0.01	1.32	1.69
belimumab	10.5	60.0	800.0	10.5	9.3	0.13	0.00	0.8	8.6	-0.03	3.61	12.23

# Twelve different biophysical assays

Code	Name	Purpose
AC-SINS	Affinity-capture self-interaction nanoparticle spectroscopy	Self-interaction
CSI	Clone self-interaction by biolayer interferometry	Self-interaction
PSR	Poly-specificity reagent	Cross-interaction
BVP	Baculovirus particle	Cross-interaction
CIC	Cross-interaction chromatography	Cross-interaction
ELISA	Enzyme-linked immunosorbent assay with commonly used antigens	Cross-interaction

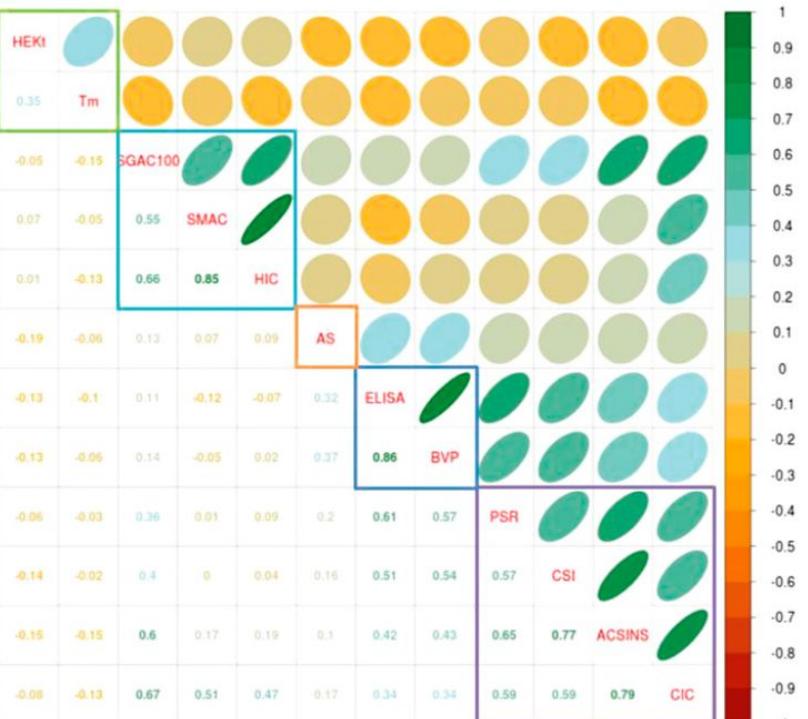
Code	Name	Purpose
HEK	Expression titer in HEK cells	Expression
Tm	Melting temperature	Thermostability
HIC	Hydrophobic interaction chromatography	Species separation and analysis
SAGC-SINS	salt-gradient affinity-capture self-interaction nanoparticle spectroscopy	Species separation and analysis
SMAC	standup monolayer adsorption chromatography	Developability
AS	Size-exclusion chromatography in accelerated stability	Stability

# Distribution of results from biophysical assays for 137 monoclonal antibodies

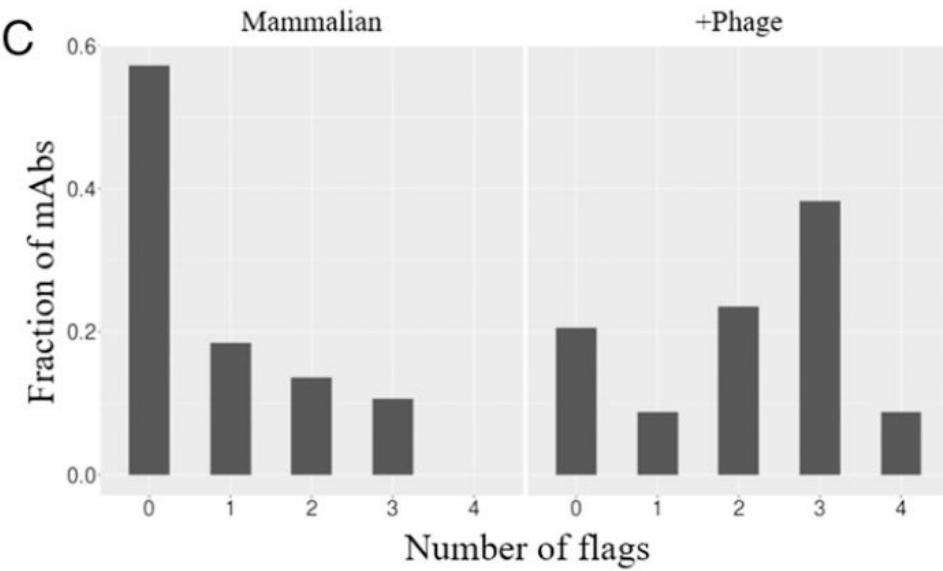
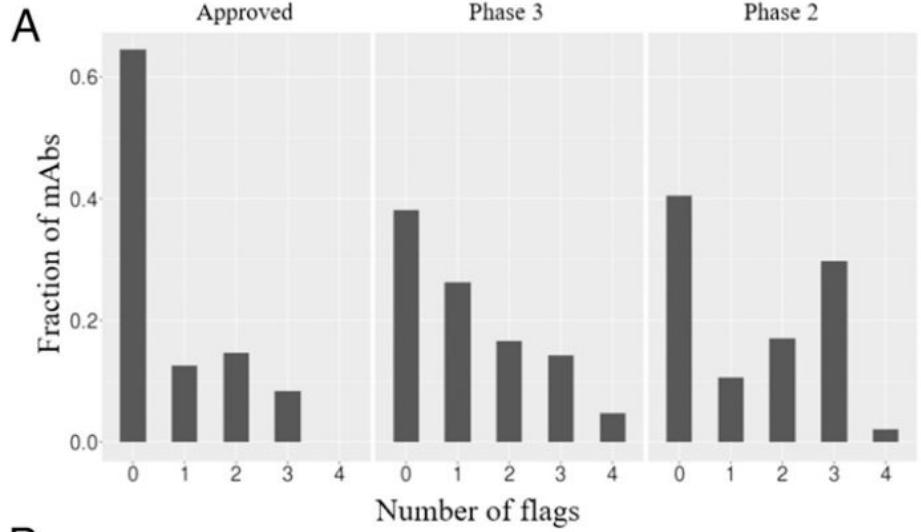


# Unsupervised clustering analysis reveals related assays

Group	Assay	Worst 10% threshold
Group 1	PSR	$0.27 \pm 0.06$
	ACSINS	$11.8 \pm 6.2$
	CSI	$0.01 \pm 0.02$
	CIC	$10.1 \pm 0.5$
Group 2	HIC	$11.7 \pm 0.6$
	SMAC	$12.8 \pm 1.2$
	SGAC-SINS	$370 \pm 133$
	BVP	$4.3 \pm 2.2$
Group 3	ELISA	$1.9 \pm 1.0$
	AS	$0.08 \pm 0.03$



# Approved antibodies and antibodies discovery not via phage display tend to have fewer flags



**D**

# Conclusions

- Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics.
- Mathematical and computational biology
  1. reveals how drug candidate work and ranks them
  2. helps with molecule design
  3. contributes to modality selection

# References

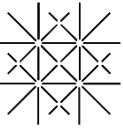
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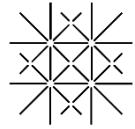


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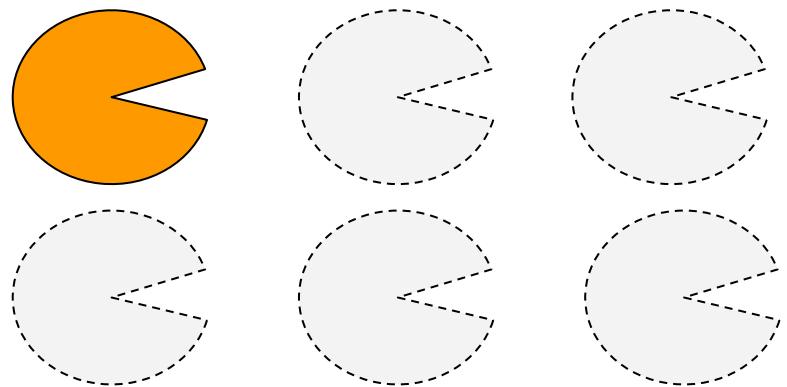
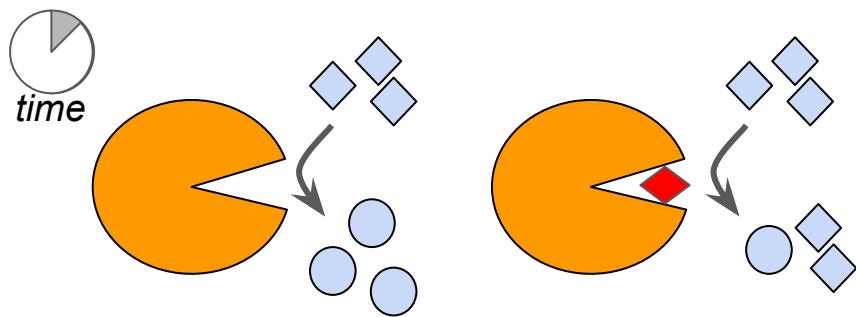


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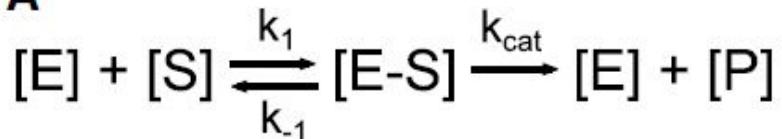
# Supplementary Information

# Competitive inhibitors reduce reaction rate; antisense oligonucleotides modulate protein abundance



# Enzymic and genetic inhibition have distinct impact on reaction dynamics

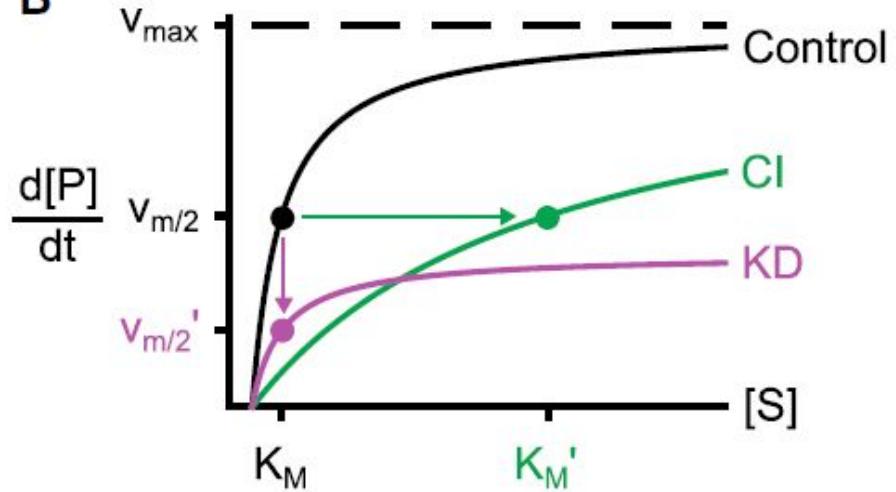
A



$$\frac{d[P]}{dt} = v_{\max} \frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1} \quad v_{\max} = k_{\text{cat}}[E]_o$$

B

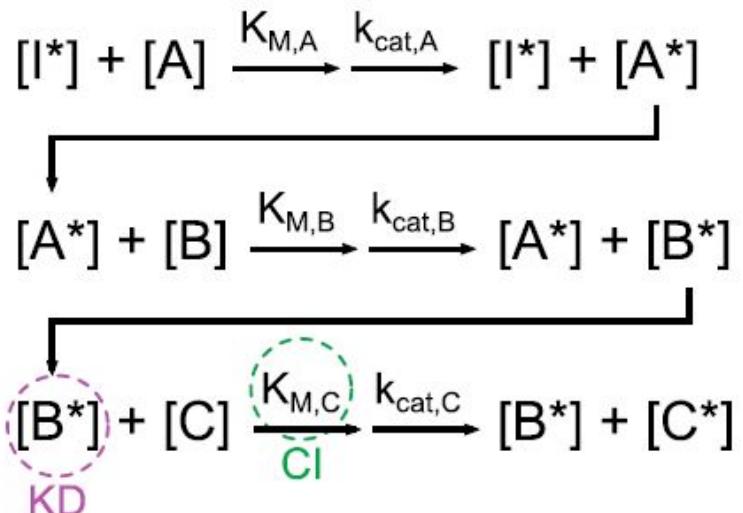


The Michaelis-Menten Equation

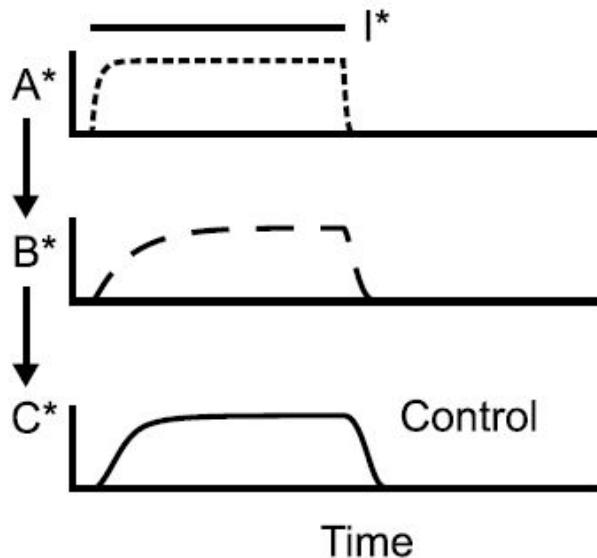
Competitive inhibition (CI)  
versus knockdown (KD)

# A linear system simulating enzymatic reactions

C



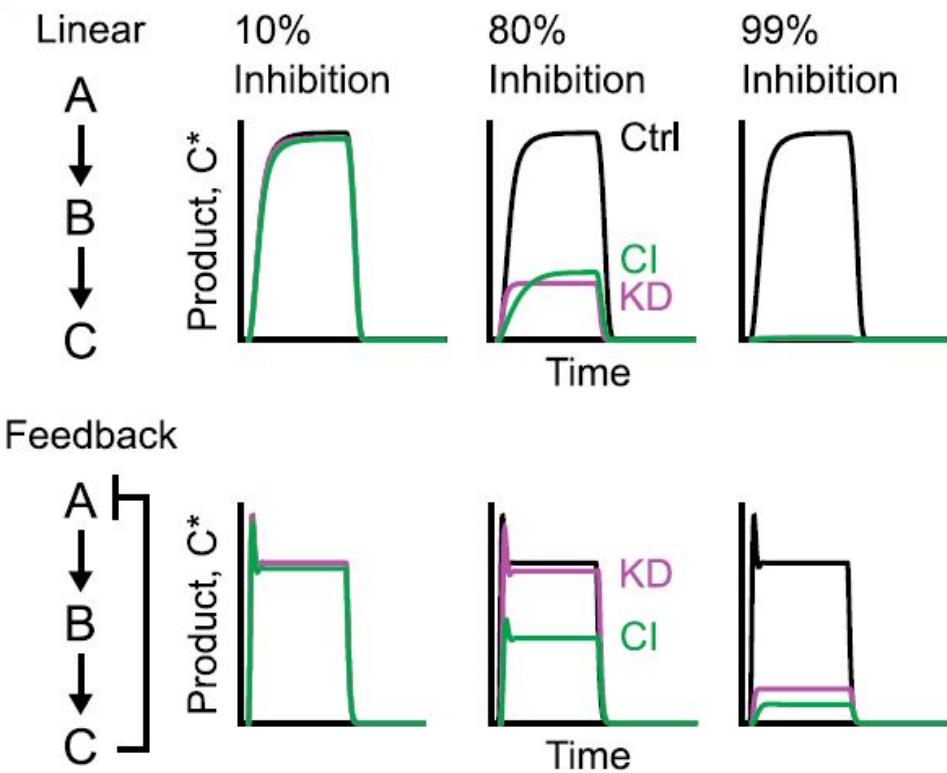
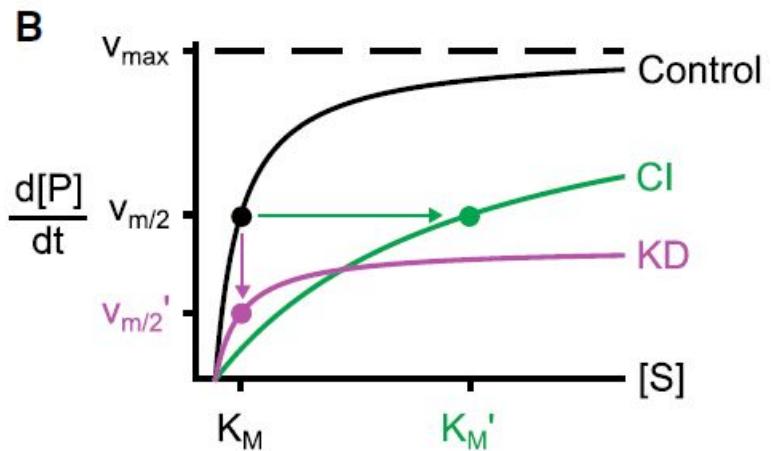
D



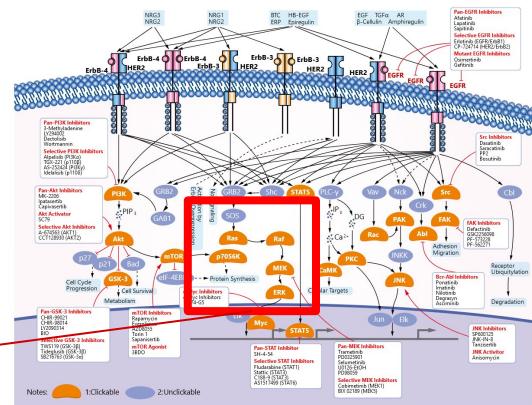
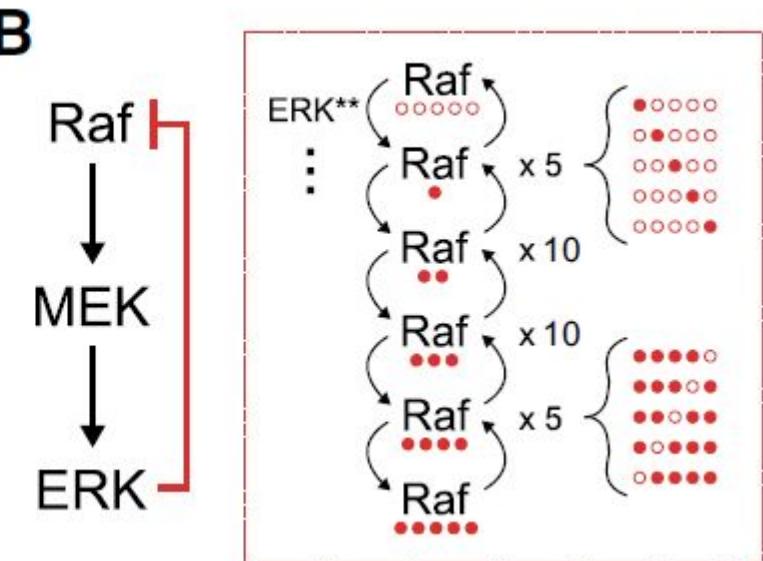
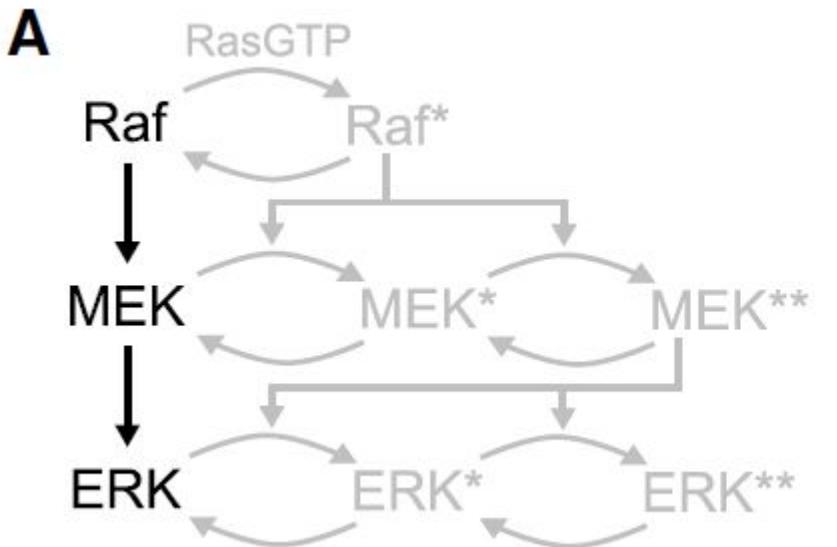
$I^*$ : upstream input;  $A/A^*$  and  $B/B^*$ : inactivated and activated enzyme;  $C^*$ : product

# Adding a negative feedback may differentiate effects of enzymatic and genetic inhibition

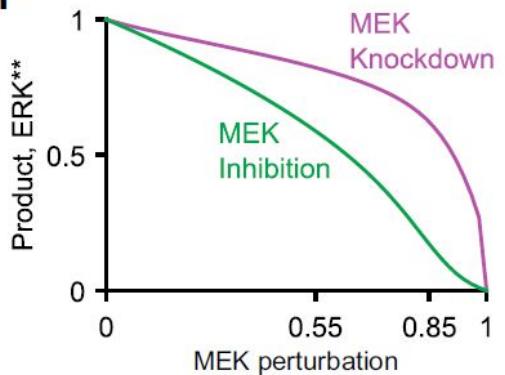
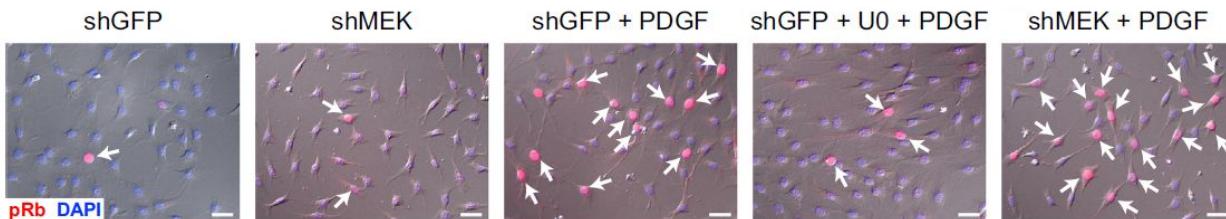
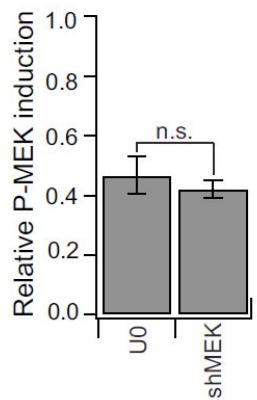
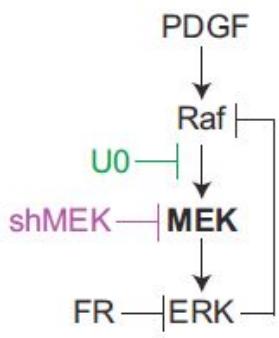
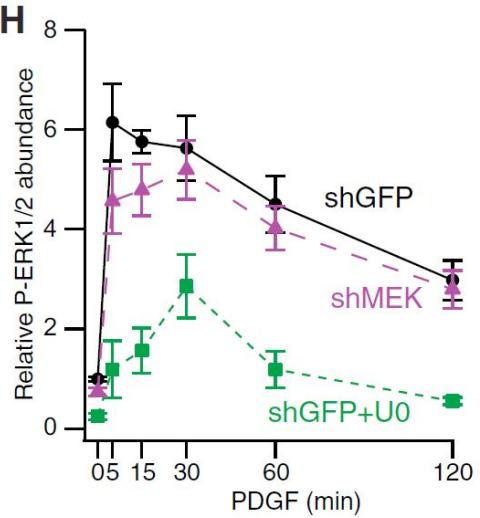
Intuition: when  $[B^*]$  stays low, CI leads to **slower** accumulation of  $C^*$  than KD.



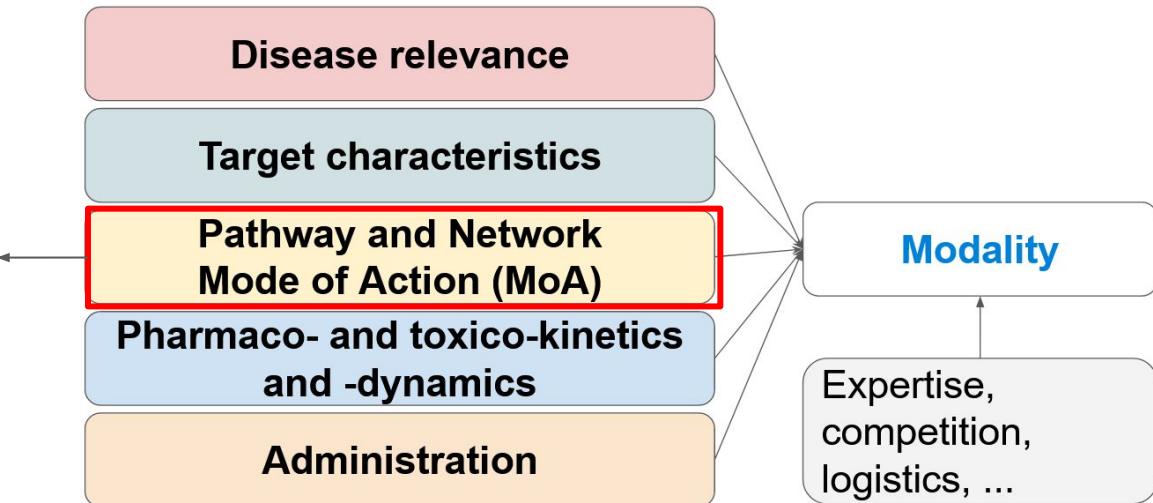
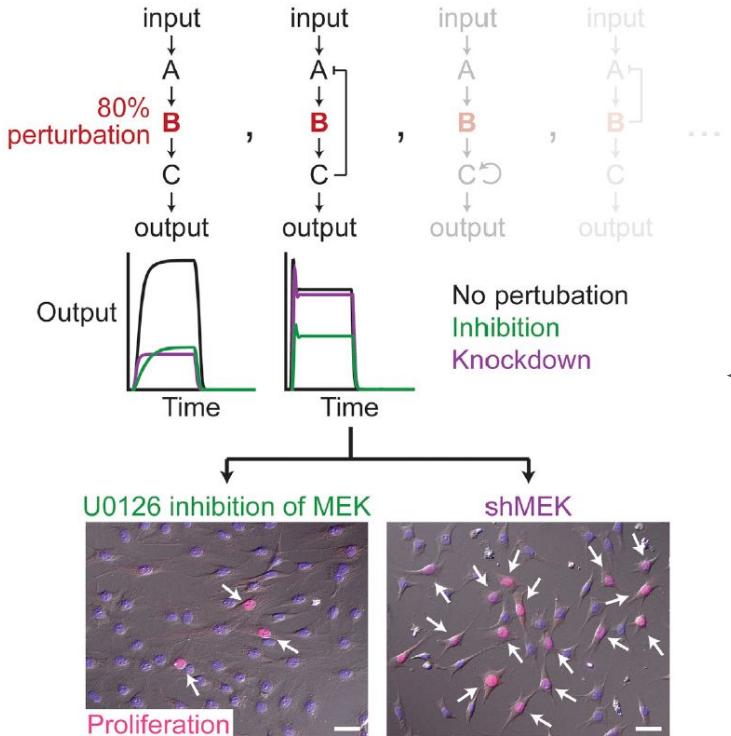
# The MAPK/ERK pathway downstream of EGFR signalling



# Confirmation of predicted difference of KD and CI

**F**

**H**


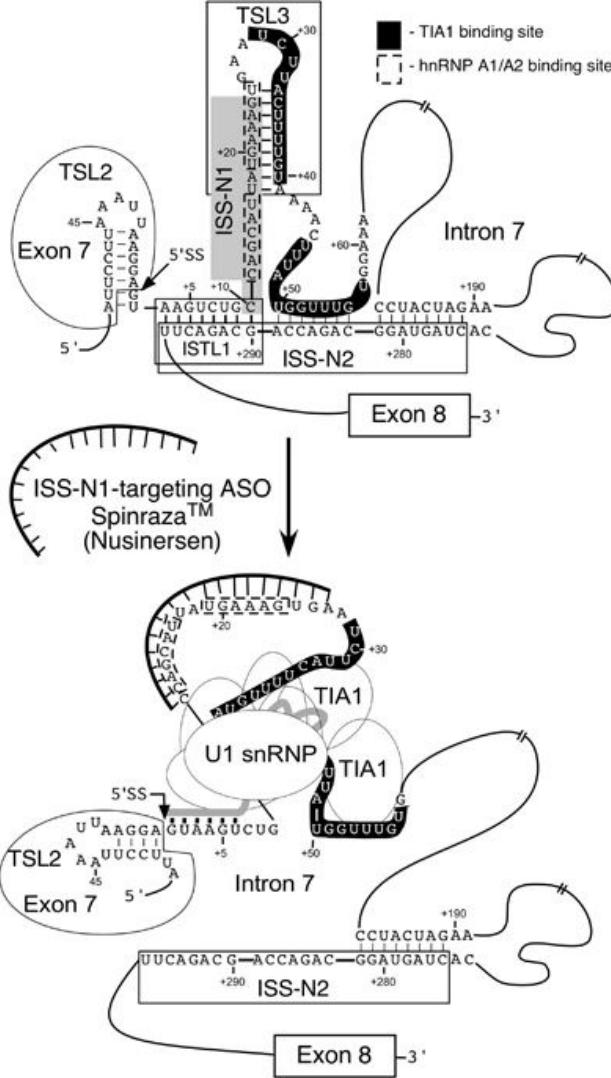
# Computational biology may empower our choice of modality



# How Spinaraza (nusinersen) works, base by base

Nusinersen binds to ISS-N1, causing structural rearrangement and recruitment of U1 snRNP by TIA1.

- ISS-N1: Intronic splicing silencer N1;
- TIA1: TIA1 cytotoxic granule associated RNA binding protein;
- TSLs: (inhibitory) terminal stem-loop structures;
- ISTL1: internal stem formed by a long-distance interaction



# Clinical-stage siRNAs

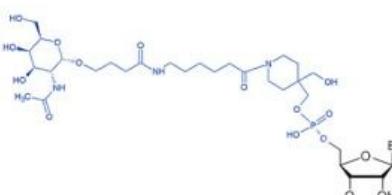
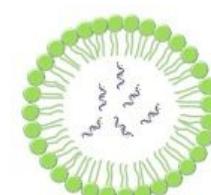
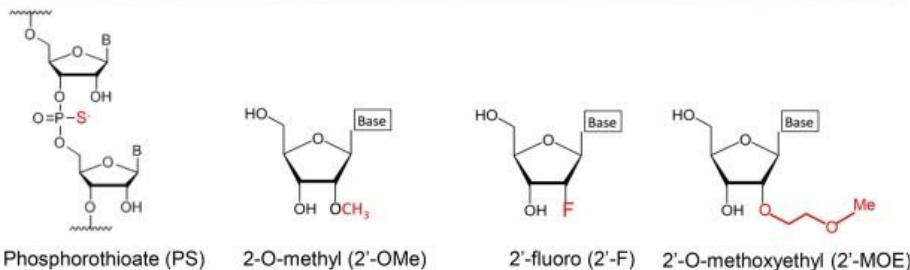
# The growth of siRNA-based therapeutics

FDA-approved siRNA drugs	Patisiran	Givosiran	Lumasiran	
siRNA drugs in clinical trials	Vutrisiran	Nedosiran	Inclisiran	Fitusiran
	Teprasiran	Cosdosiran	Tivanisiran	
Drug	Alternative name	Company	Disease	Updated status
Patisiran	ONPATTRO	Alynlam	Hereditary transthyretin mediated amyloidosis	FDA approval in 10/08/2018 210922Orig1s000*
Givosiran	GIVLAARI	Alynlam	Acute hepatic porphyria	FDA approval in 11/20/2019 212194Orig1s000
Lumasiran	ALN-GO1	Alynlam	Primary hyperoxaluria type 1 (PH1)	FDA approval on 11/23/2020 214103Orig1s000
Vutrisiran	ALN-TTRsc02	Alynlam	Hereditary transthyretin mediated amyloidosis	Phase 3 trials ELIOS-A (NCT03759379)** HELIOS-B (NCT04153149)
Nedosiran	DCR-PHXC	Dicerna Alynlam	Primary hyperoxaluria	Phase 3 trial PHYOX 3 (NCT04042402)
Inclisiran	ALN-PCSSC	Alynlam Novartis	Hypercholesterolemia	Phase 3 trials ORION-9 (NCT03397121) ORION-10 (NCT03399370) ORION-11 (NCT03400800)
Fitusiran	ALN-AT3sc ALN-APC SAR439774	Alynlam Sanofi Genzyme	Hemophilia A and B	Phase 3 trials ATLAS-A/B (NCT03417245) ATLAS-INH (NCT03417102) ATLAS-PPX (NCT03549871) ATLAS-PEDS (NCT03974113) ATLAS-OLE (NCT03754790)
Teprasiran	AKII-5, DGF1, I-5NP, QPI-1002	Quark Novartis	Acute kidney injury Delayed graft function	Phase 3 trial ReGIFT (NCT02610296)
Cosdosiran	QPI-1007	Quark	Non-arteritic anterior ischemic optic neuropathy (NAION)	Phase 2/3 trial NCT02341560
Tivanisiran	SYL-1001	Sylentis	Dry eyes Ocular pain	Phase 3 trial HELIX (NCT03108664)

\* FDA application number.

\*\* ClinicalTrials.gov identifier number at <https://clinicaltrials.gov/ct2>

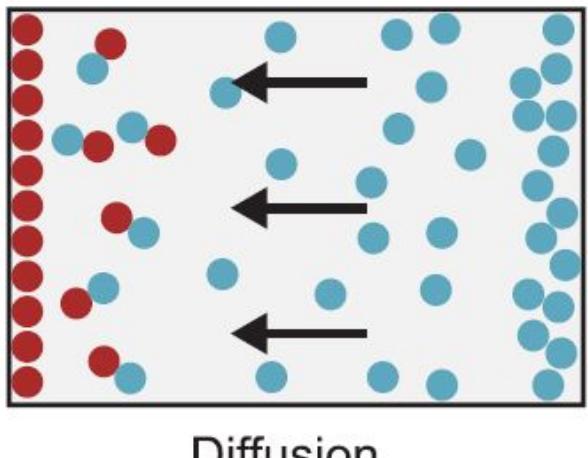
Drug	Backbone	Chemical modifications			Delivery platform
		PS	2'-OMe	2'-F	
Patisiran	-	+ (11)	-	-	LNP
Givosiran	+ (6)	+ (28)	+ (16)	-	GalNAc
Lumasiran	+ (6)	+ (34)	+ (10)	-	GalNAc
Vutrisiran	+ (6)	+ (35)	+ (9)	-	GalNAc
Nedosiran	+ (6)	+ (35)	+ (19)	-	GalNAc
Inclisiran	+ (6)	+ (32)	+ (11)	+ (1)	GalNAc
Fitusiran	+ (6)	+ (23)	+ (21)	-	GalNAc
Teprasiran	-	+ (19)	-	-	None
Cosdosiran	-	+ (9)	-	-	None
Tivanisiran	-	-	-	-	None



# Chemically induced proximity

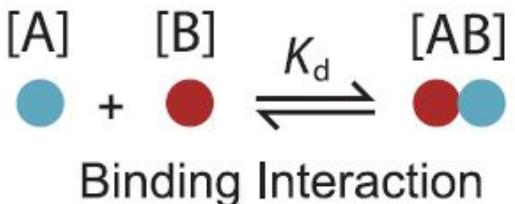
# A reaction-diffusion model

Reaction-Diffusion System



$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} + ku$$

Diffusion      Binding



*x*: position

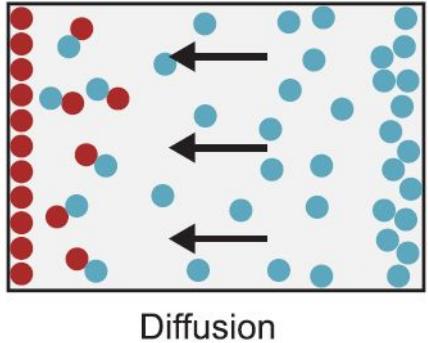
*u*: product concentration

*t*: time

The diffusion term follows *Fick's second law of diffusion*; the binding term describes the reaction.

# Kinetic and thermodynamic contributions of chemically induced proximity

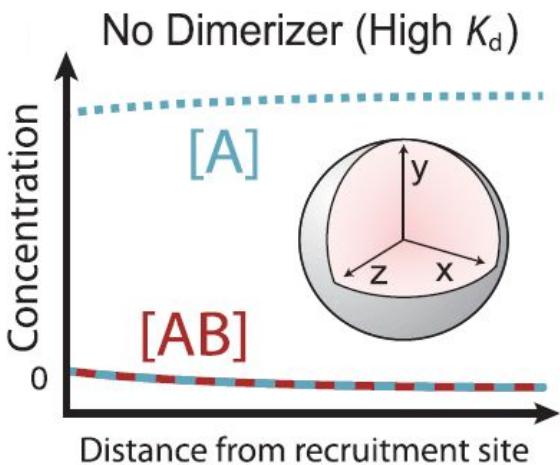
Reaction-Diffusion System



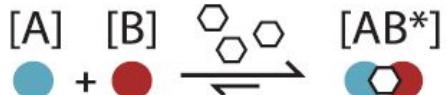
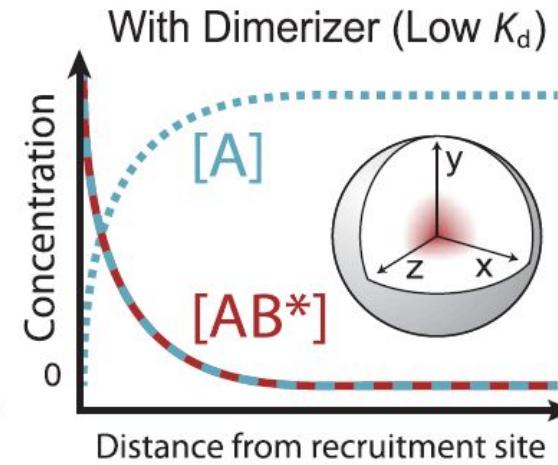
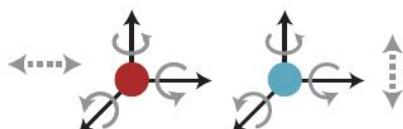
$$\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} + ku$$



Binding Interaction



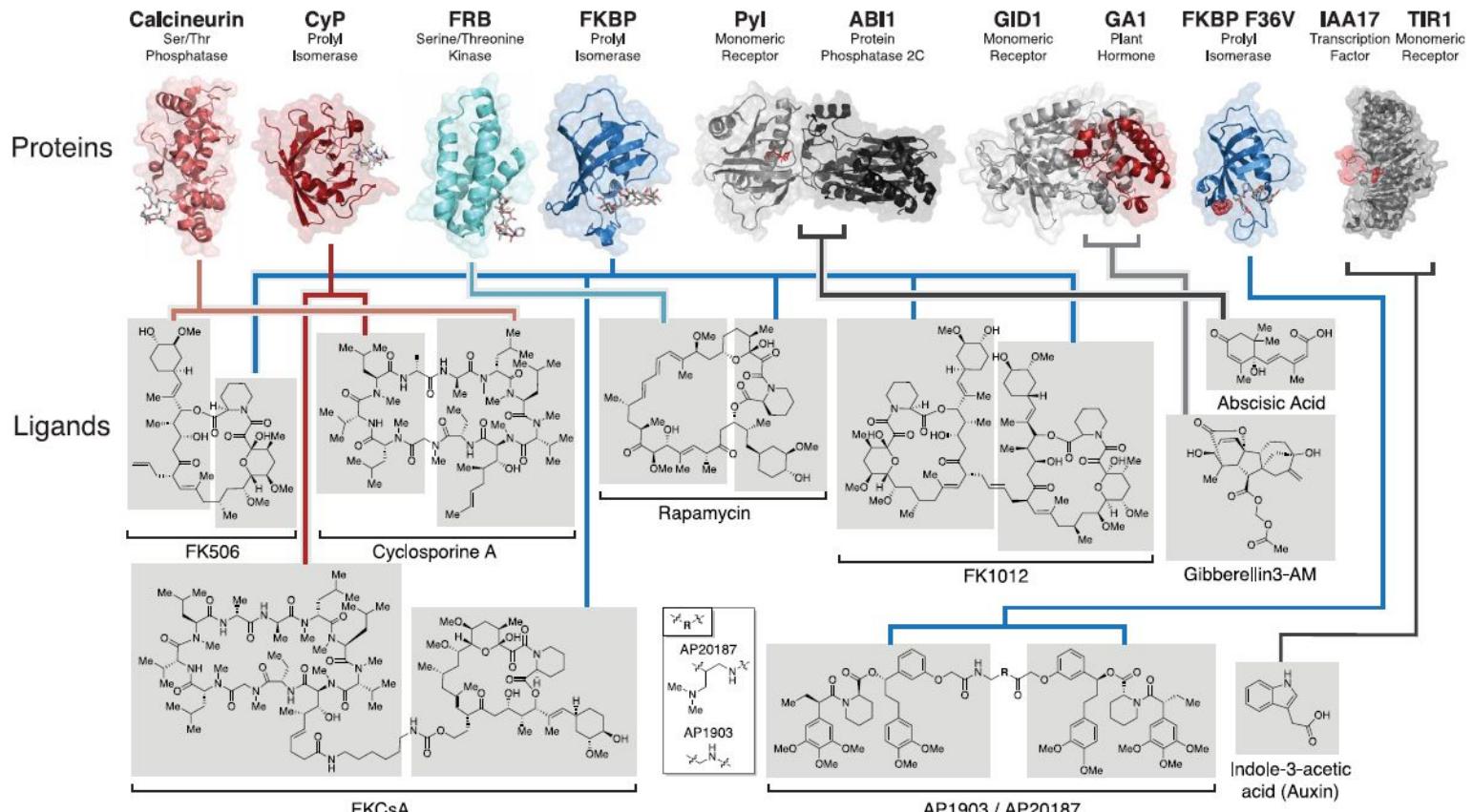
Freely Diffusing



Constrained upon Dimerization

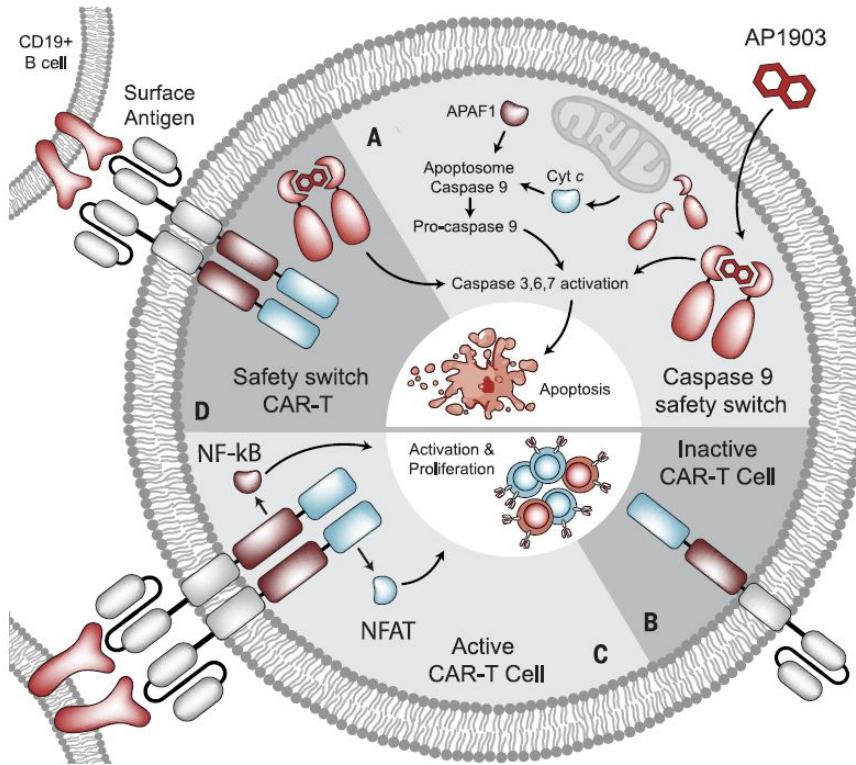


# Chemically induced proximity



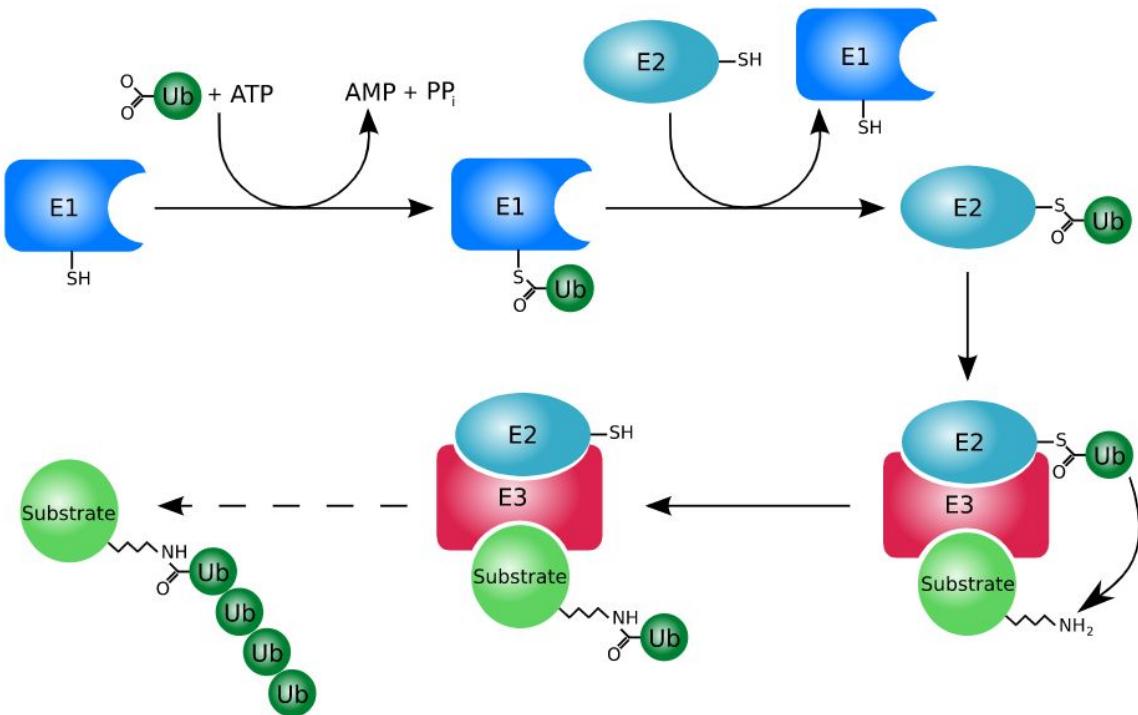
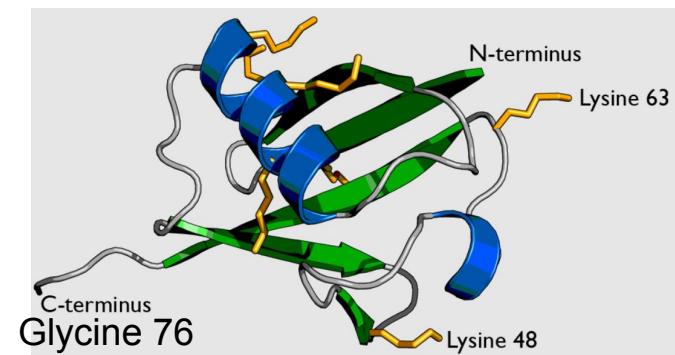
# Chemically induced proximity as ‘safety switch’ for cell therapy

- Too many or too active CAR-T cells may induce serious side effects (cytokine release syndrome, B cell aplasia, etc.)
- *Bioinert* small molecules (AP1903 in this case) can be used as ‘safety switch’ to kill transplanted CAR-T cells.



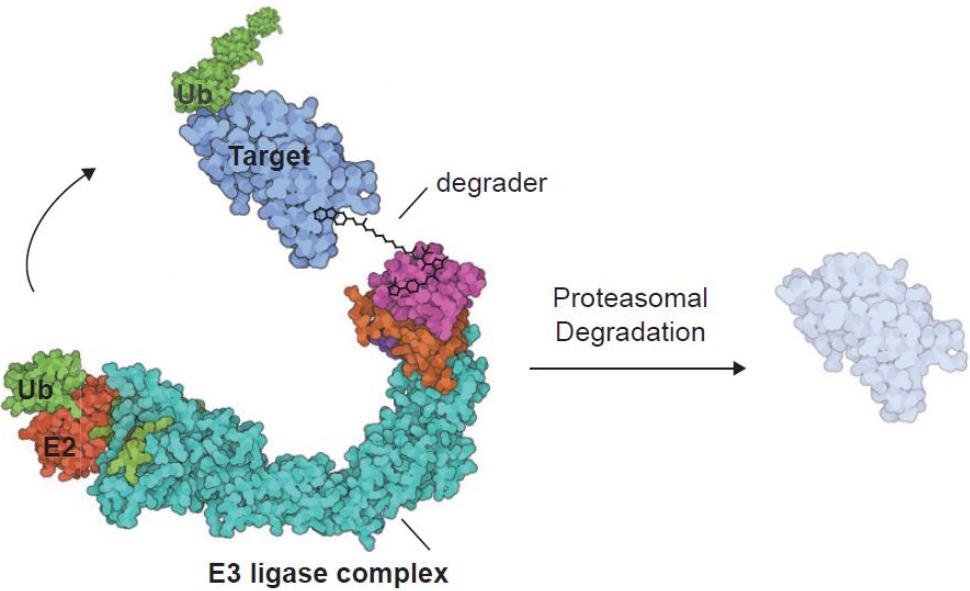
# Therapeutic use of protein degradation

# Ubiquitination marks proteins to be degraded

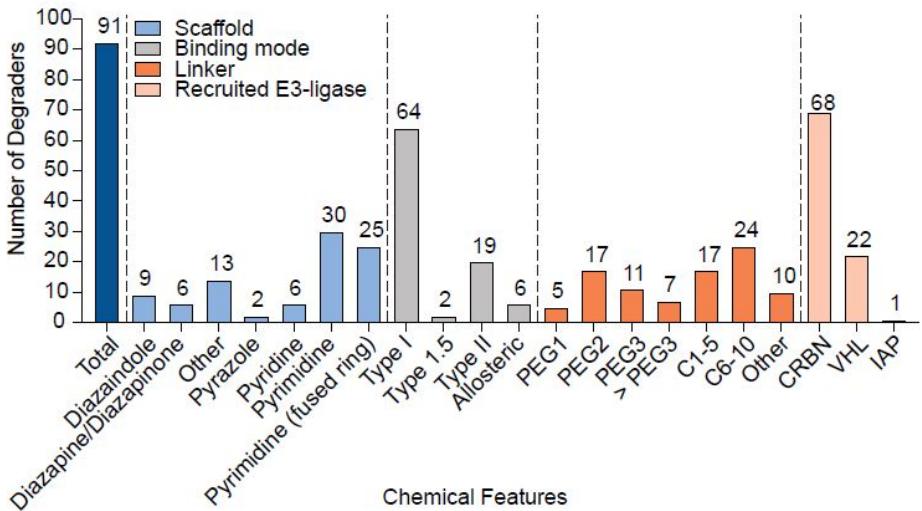
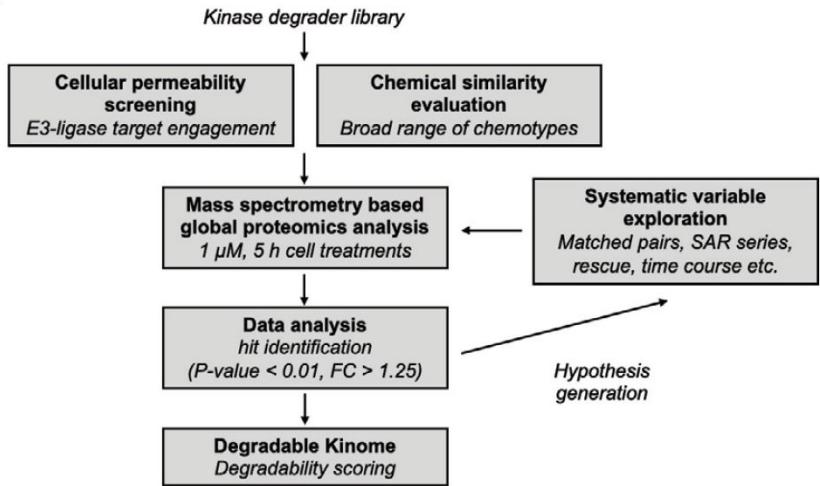


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# Donovan et al. (2020) reports screening results with 91 kinase degraders

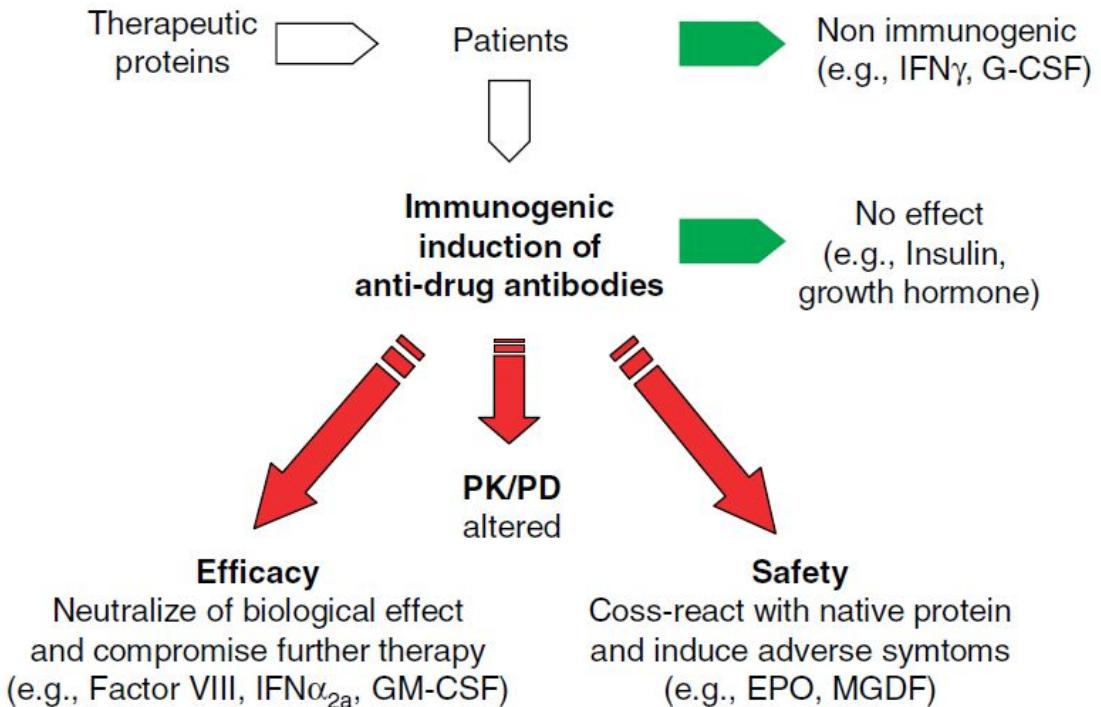


# Donovan et al. (2020) reports screening results with 91 kinase degraders

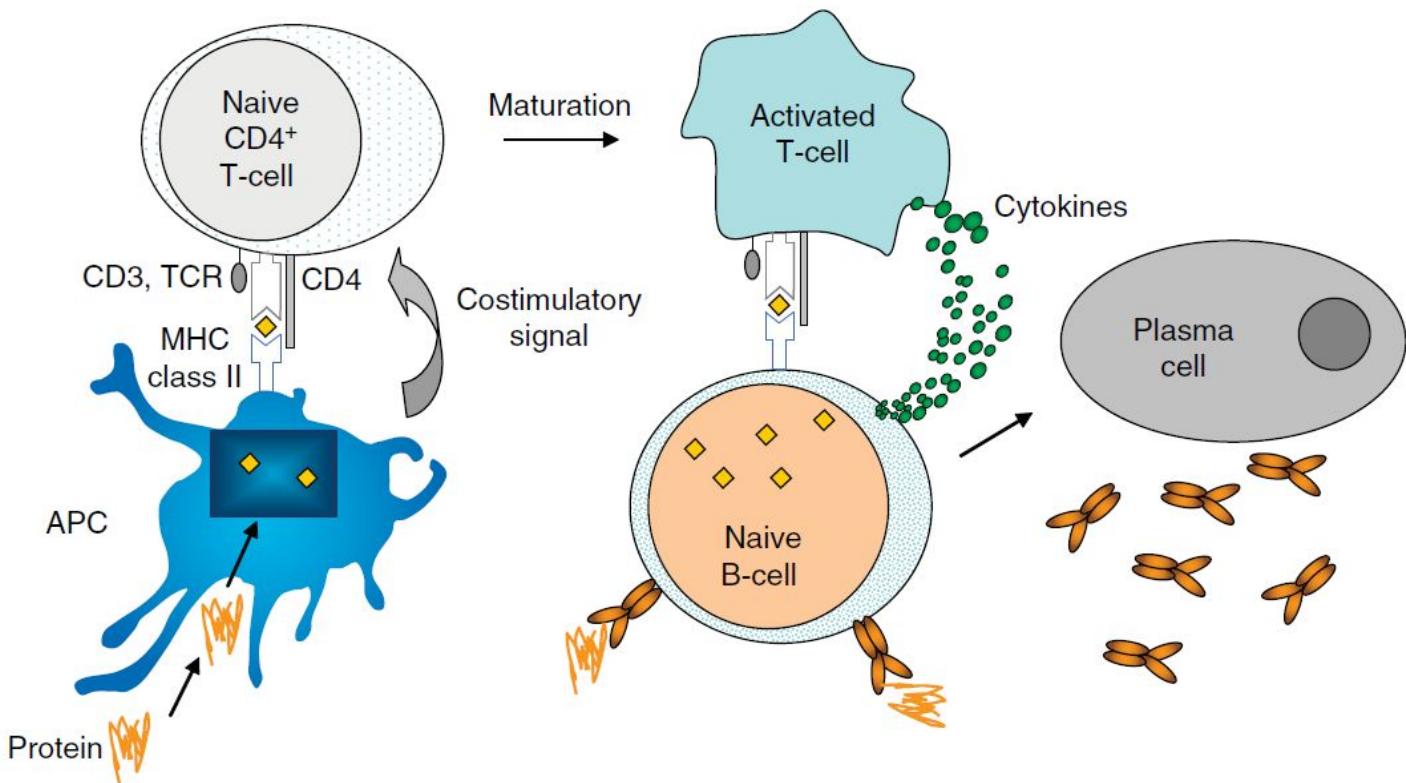


# Immunogenicity of therapeutic proteins

# Immunogenicity affects both efficacy and safety



# Immune response underlies immunogenicity

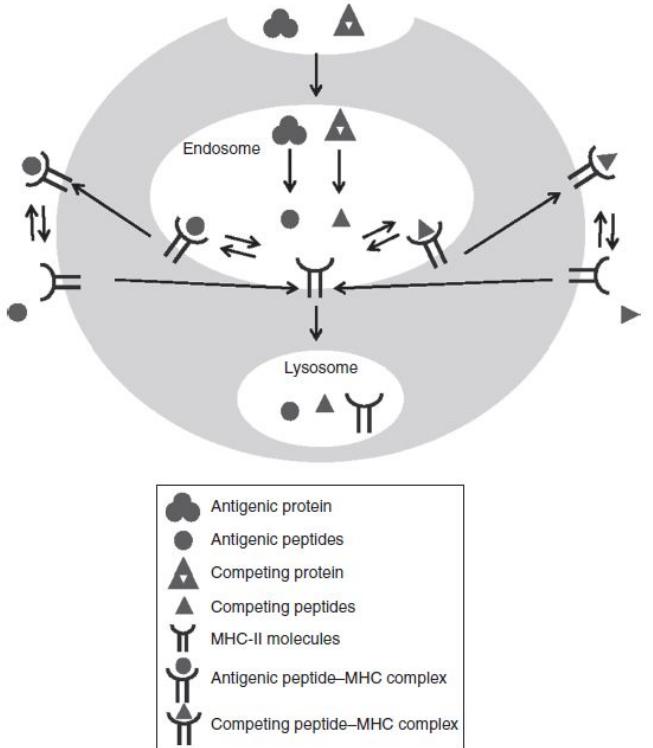


**Table 2. The factors contributing to immunogenicity are divided into three groups.**

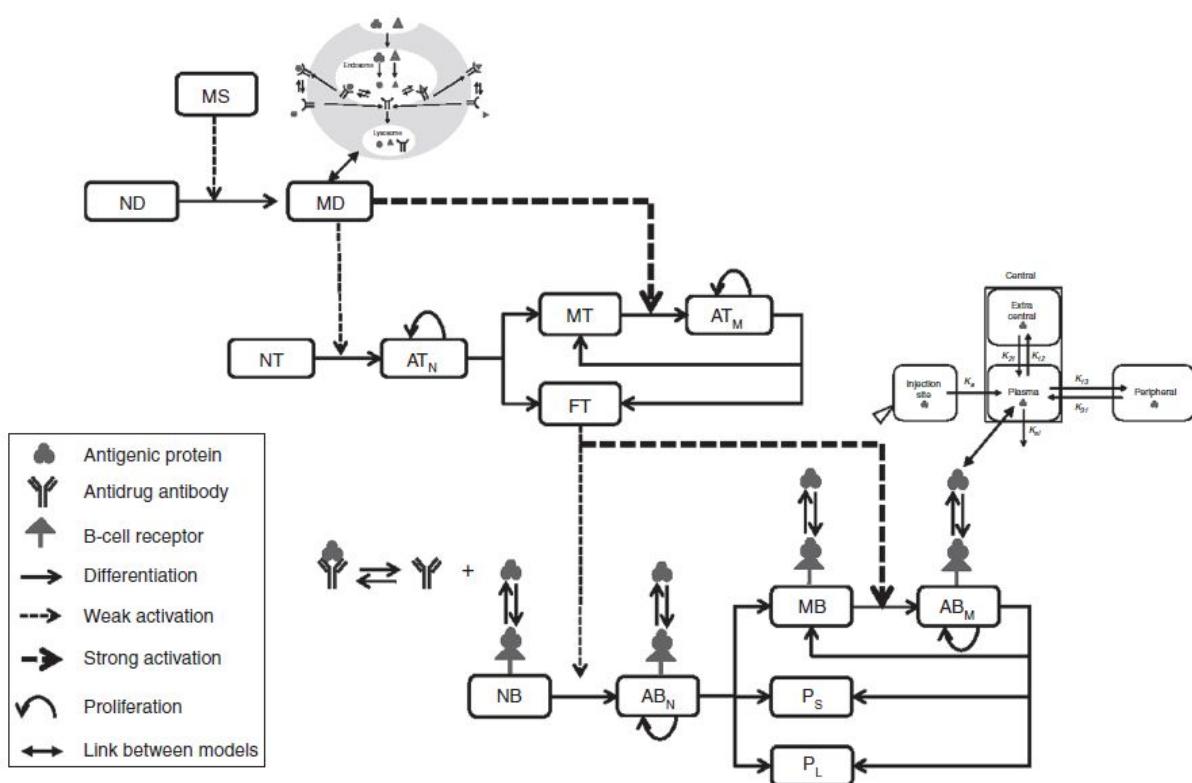
	<b>Immunogenicity potential</b>
<i>Drug product</i>	
Host	Non-human: ▲
Immunomodulatory properties	▲?
Glycosylation	▲
Aggregation	▲
Size	Molecular mass < 10 kDa: ▼
Formulation	Polymers ▼
Excipients/stabilizers	To be characterized: silicone oil ▲
Impurities	Product/process related: ▲
Post-translational mod.	Oxidation, deamidation, etc.:▲
aa Composition	Charged aa: ▲; Aromatic aa: ▼
Conjugates	▲
<i>Patient</i>	
Age	▼?
Disease state	Different indication/different response
Immune status	Immune compromised: ▼ Infective disease: ▲
Patient to patient variability	Not predictable
Concomitant therapy	Earlier exposure to similar protein - crossreacting antibodies to similar proteins
Genetic factors	Defective gene Polymorphisms for cytokines
<i>Administration</i>	
Dose	Higher dose: ▲?
Route	Intravenous administration less immunogenic than subcutaneous or intramuscular Short-term administration less immunogenic than long-term treatment Continuous administration less immunogenic than intermittent
Frequency	More frequent: ▲
Duration of therapy	Short term: ▼

▲: Potential to increase immunogenicity; ▼: Potential to decrease immunogenicity; ?: Most likely; aa: Amino acid.

# A mechanistic, multiscale model of immunogenicity: subcellular model

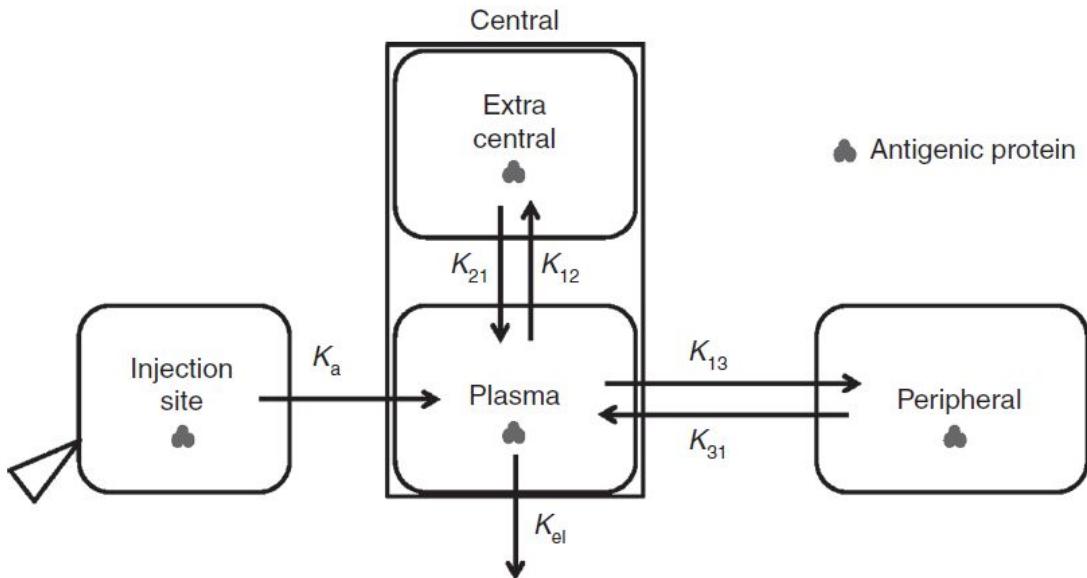


**Figure 1.** Model structure for the subcellular level, including processes for antigen presentation in mature dendritic cells. The symbols in the figure legends are described below, with corresponding equation number in **Supplementary Materials** shown between parentheses. ●: Antigenic protein, including antigenic protein in plasma ( $Ag$ , Eq. 27 in **Supplementary Material**) and antigenic protein in the endosome: ( $Ag^E$ , Eq. 4 in **Supplementary Material**); ●: antigenic peptide in endosome ( $p_j^E$ , Eq. 5 in **Supplementary Material**); ▲: competing protein in the endosome ( $cp^E$ , Eq. 9 in **Supplementary Material**); ▲: competing peptide in the endosome ( $cpt^E$ , Eq. 10 in **Supplementary Material**); ¶: MHC-II molecules, including those in the endosome ( $M^E_k$ , Eq. 6 in **Supplementary Material**) and those on dendritic cell membrane ( $M_k$ , Eq. 13 in **Supplementary Material**); ¶: antigenic peptide–MHC complex, including those in the endosome ( $pM^E_k$ , Eq. 7 in **Supplementary Material**) and those on cell membrane ( $pM_k$ , Eq. 8 in **Supplementary Material**); ¶: competing peptide–MHC complex, including those in the endosome ( $cptM^E_k$ , Eq. 11 in **Supplementary Material**) and those on cell membrane ( $cptM_k$ , Eq. 12 in **Supplementary Material**).

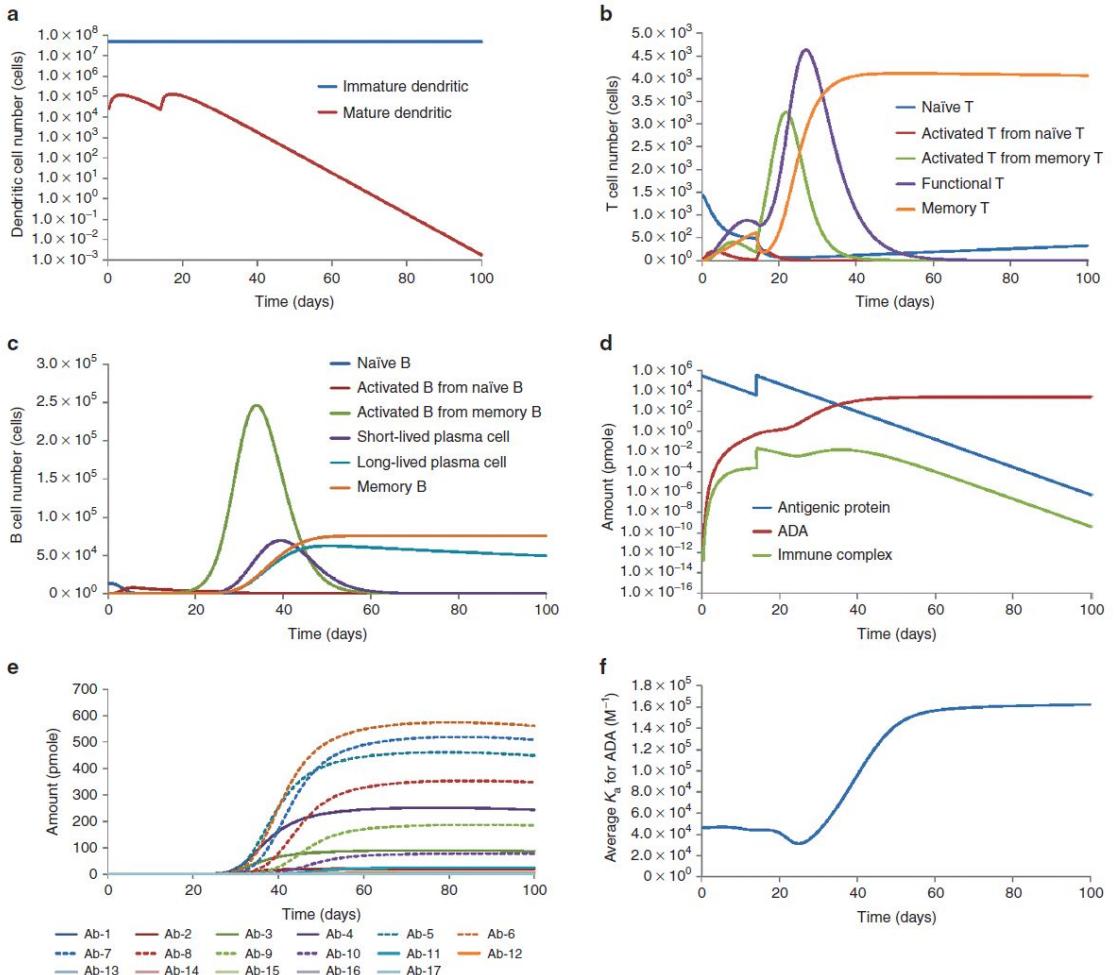


**Figure 2.** Model structure for the cellular level, including cells, antigen, antidrug antibody, and B-cell receptor. The links between the three levels of the multiscale model are also illustrated to help interpretation. The acronyms are explained below, along with the corresponding equation number in the Supplementary Material shown between parentheses. MS: maturation signal (Eq. 1 in Supplementary Material); ID: immature dendritic (Eq. 2 in Supplementary Material); MD: mature dendritic (Eq. 3 in Supplementary Material); NT: naïve T (Eq. 14 in Supplementary Material); AT<sub>N</sub>: activated T from naïve T (Eq. 15 in Supplementary Material); AT<sub>M</sub>: activated T from memory T (Eq. 16 in Supplementary Material); MT: memory T (Eq. 17 in Supplementary Material); FT: functional T (Eq. 18 in Supplementary Material); NB: naïve B (Eq. 19 in Supplementary Material); AB<sub>N</sub>: activated B from naïve B (Eq. 20 in Supplementary Material); AB<sub>M</sub>: activated B from memory B (Eq. 21 in Supplementary Material); MB: memory B (Eq. 22 in Supplementary Material); P<sub>S</sub>: short-lived plasma (Eq. 23 in Supplementary Material); P<sub>L</sub>: long-lived plasma cell (Eq. 24 in Supplementary Material).

# The whole-body model

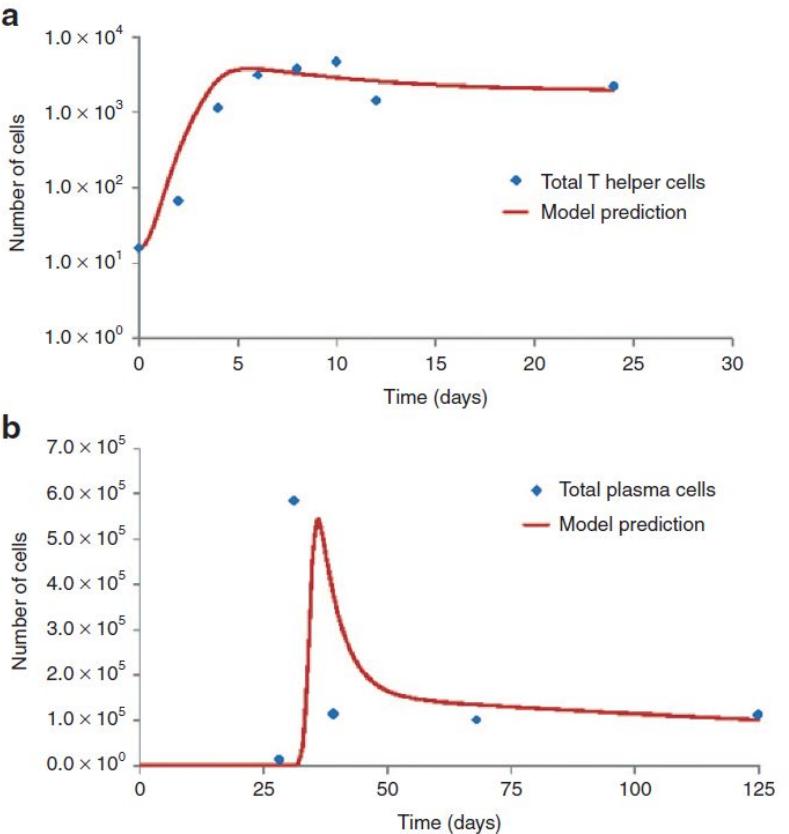


**Figure 3.** Model structure for the whole-body level, accounting for the *in vivo* disposition of antigenic protein. Details are described in the Results section and also by **Eqs. 26–29 in the Supplementary Materials.**



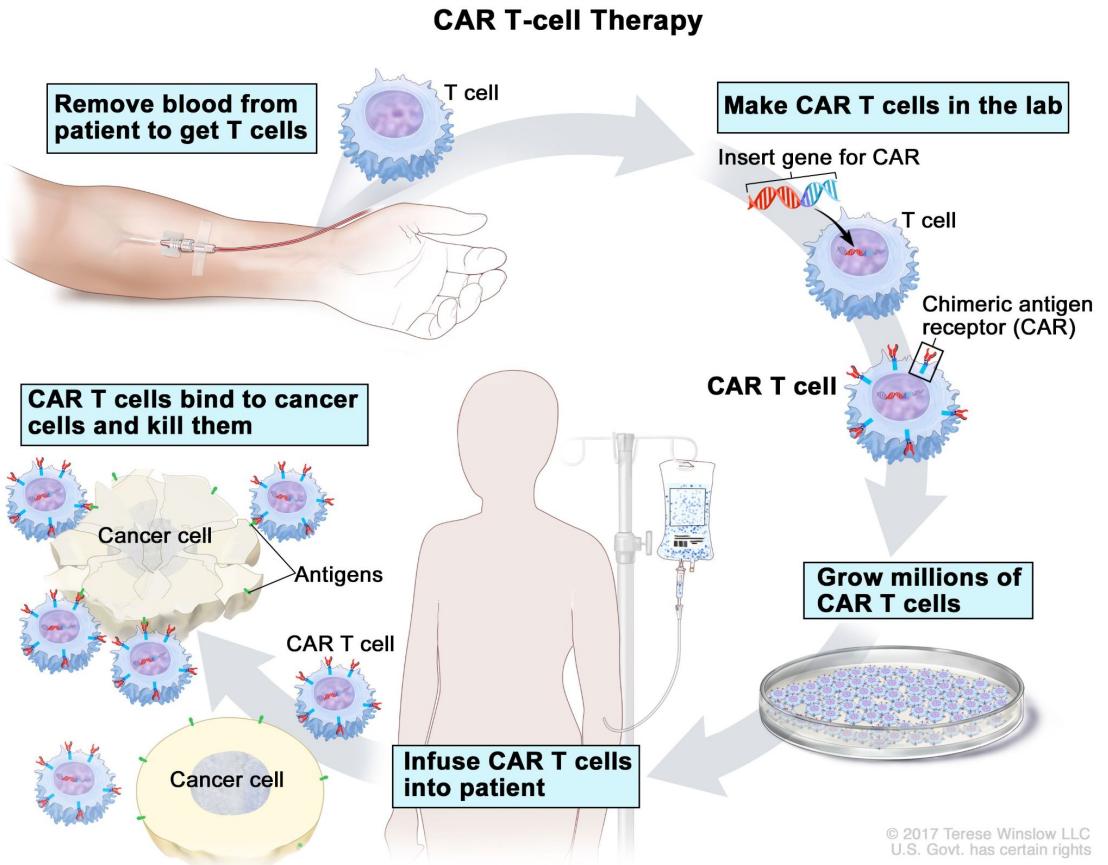
**Figure 4. Simulation results of immune responses in human against a theoretical antigenic protein.** The results include kinetic profiles for (a) dendritic cells; (b) helper T cells; (c) B cells; (d) antigenic protein, ADA, and immune complex; (e) polyclonal ADA (total 17 clones, whose antigen-binding affinity increases by twofold between clones, from clone 1 to clone 17); (f) average antigen-binding affinity of ADA. ADA, antidiug antibody.

# Observation and model prediction

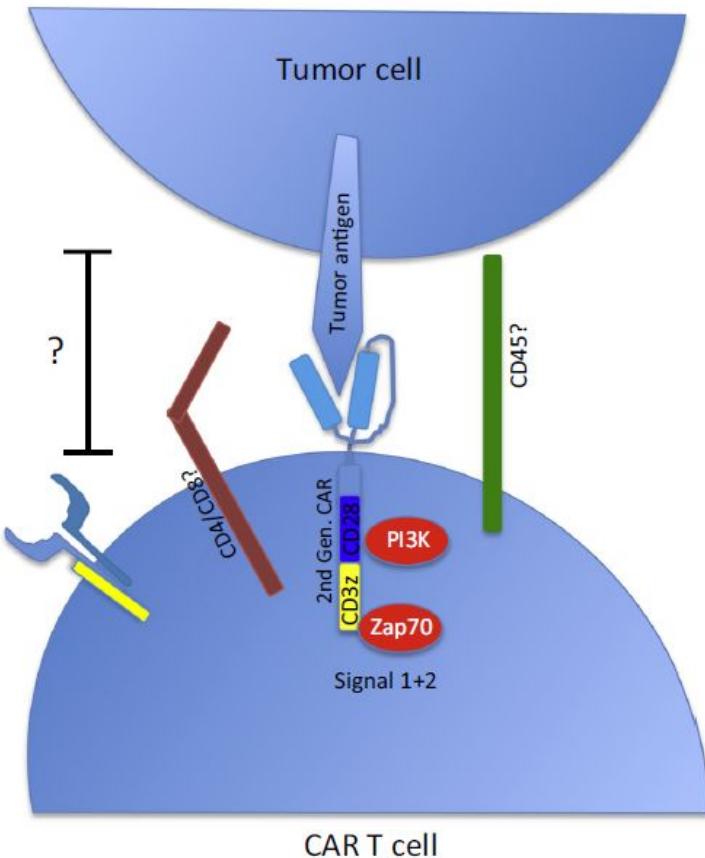
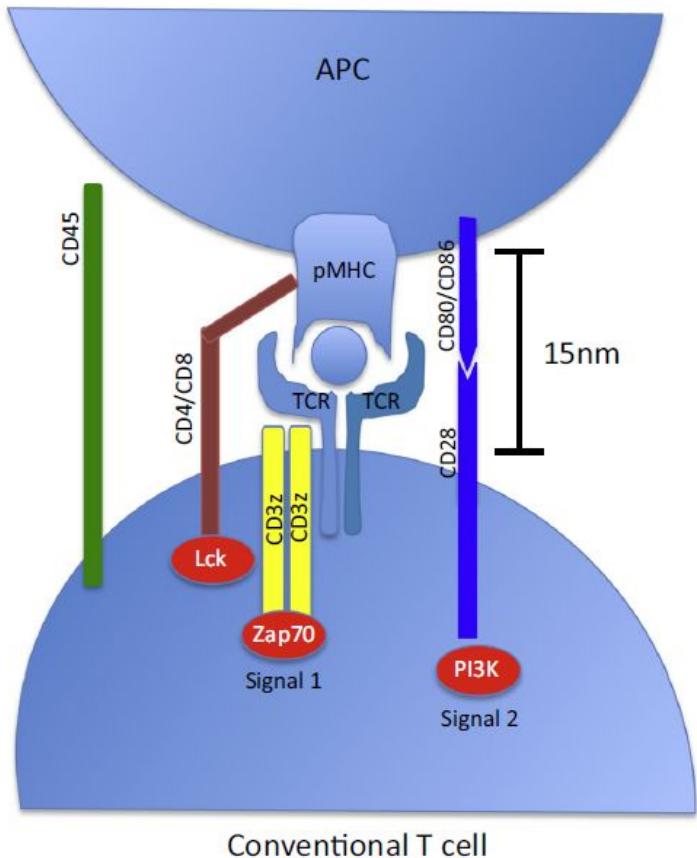


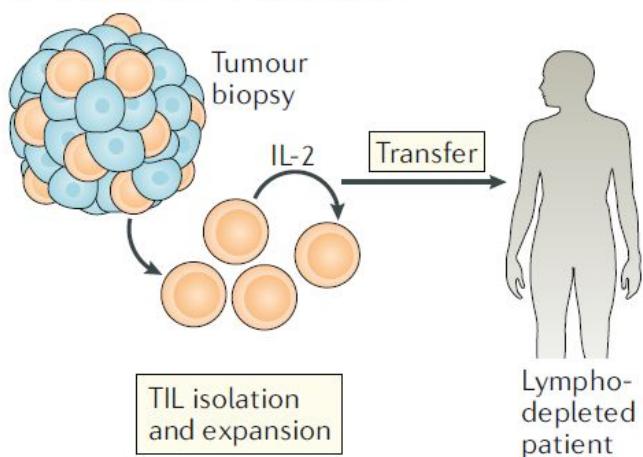
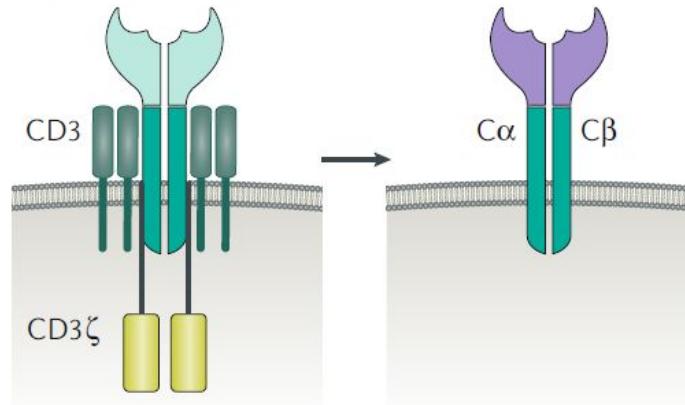
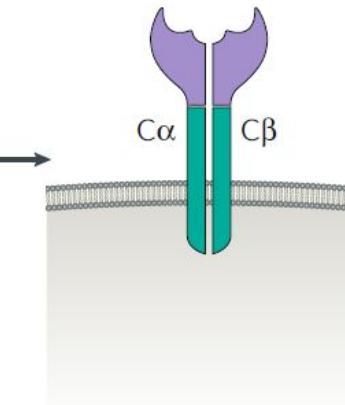
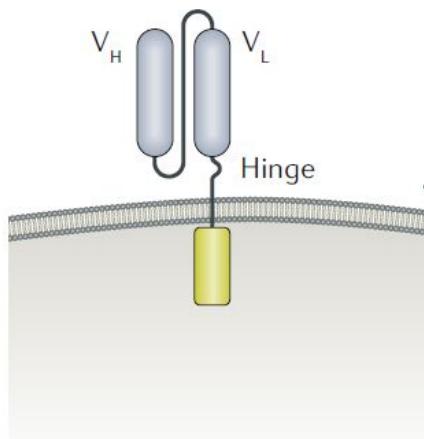
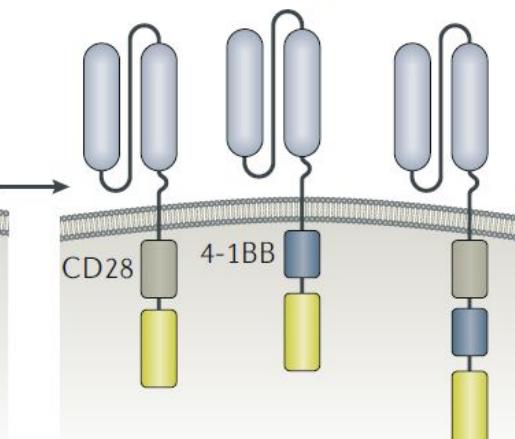
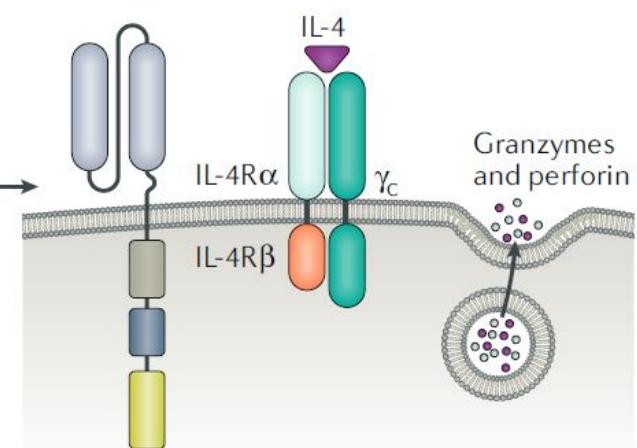
# CAR-T and individualized vaccines

# CAR (Chimeric Antigen Receptor) T-Cell Therapy



# Signaling of conventional and CAR T cells



**a TAA-specific T cell transfer**

**b Physiological TCR-CD3 complex**

**Recombinant TCR**

**c First-generation CAR**

**Second- and third-generation CARs**

**Fourth-generation CAR**


# Towards personalized vaccine development

